THE
AMERICAN NATURALIST

Vol. LXXXVII July-August, 1953 No. 835

SURFACE-SPREAD PROTEIN AS A BASIS FOR CELL STRUCTURE AND CELL MOVEMENT*

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INTRODUCTION

As Inoué (1952) has shown us by means of his development of the polarized light technique, much new information is now available pointing to the physical organization of the mitotic apparatus and the changes involved in this cellular structure during the process of cell division. It is not my purpose to propose any new hypothesis as to the mechanism involved in these changes, or in any other changes involved in the process of cell division, such as chromosomal movement of cytokinesis. The wealth of observations, not only of the various phases of ordinary mitosis, but the cases of unusual variations of the mitotic process, so ably summarized by lifelong students of this basic phenomenon such as Schrader (1944), Hughes (1952), and others, make it all too obvious that any simple explanation will not suffice. I should like, however, to consider the question from the standpoint of one who is primarily interested in the formation of, and the molecular state of, intracellular structures. Therefore, I would like to put forward for consideration a physicochemical system as the basis for cell structures, which, so far as I am aware, has not been proposed in speculations on the mechanism of mitosis. A statement by Schrader (1951) here comes to mind with peculiar aptness, for he said: "In short, we have after long years come to the realization, not altogether a happy one, that we are confronted with a very complex process, and that its analysis may call for physical and chemical information about which the physicist and chemist themselves are at present none too sure."

With this I can concur heartily, for the system I want to propose is one about which the chemist and physicist and perhaps I myself, are none too sure. Briefly stated, I would like to suggest that cellular structures are formed by, and composed of, protein or possibly lipo-protein molecules, surface-spread at the interfaces of the heterophasic system within the cell.

*Paper given at President's Symposium, "Recent Advances in our Knowledge of Mitosis." American Society of Zoologists, Ithaca, New York, September 10, 1952.

SUMMARY OF INFORMATION FROM PROTEIN SURFACE STUDIES

The first measured observation on surface films was probably that of Benjamin Franklin in 1765, who noted that a teaspoon of oil spread over the surface of a pond to the extent of a half-acre (from Sobotka, 1944). Since Franklin's time, no more studies were made until the development of modern work begun by the experiments of Devaux and Langmuir and others about the turn of this century. We now know that many substances will form films at heterophasic interfaces, and especially those organic substances such as lipids or proteins which contain both hydrophobic and hydrophilic groups. The information on *protein* behavior at air-water or oil-water interfaces may be summarized as follows:

1. Protein molecules are oriented with respect to the surface, with the polypeptide chains lying in the surface, and hydrophilic and hydrophobic side-chains pointed toward and away from the aqueous phase, respectively.

2. The molecules become physically altered at the interface. As a primary reaction, globular protein molecules become flattened so that in the plane of the interface they occupy a large area. This change is probably due to the breaking of certain bonds, and unfolding of the molecule. In the extreme state, such opened molecules have a thickness of 7.5–10 A°. Some protein molecules break up into smaller kinetic units upon interaction with the interface (Joly, 1948; Cheesman, 1952; Tonomura, 1949) whereas others do not.

2. Joly (1939) has shown that if protein is spread against a constant surface pressure above a certain minimum value, the film obtained, called "B" films, will have a greater thickness and different properties as compared to films spread against lower pressures ("A" films). The "A" films are shown to be characteristically insoluble, inert, with minimum thickness and low surface viscosity, all of which properties are quite constant and reproducible. The "B" films, on the other hand, are thicker, with a higher surface viscosity which changes with time. Joly considers the B-films to be composed of molecules either incompletely unfolded, or molecules in a state of constraint. Agreement with the first of these possibilities comes from Bateman and Chambers (1941), who interpret their results as indiçating degrees of molecular unfolding.

3. Finally, we may note that films at extremely low pressures behave as two-dimensional gases with characteristic Newtonian viscosity, and with increasing pressure and greater constraint, they pass into a liquid phase with increasing viscosity which becomes non-Newtonian. With even higher pressures, the component molecules become tightly packed together to form a solid film, and if the pressure is still further increased, the film "collapses"; that is, the solid film becomes folded with the folds perpendicular to the direction of compression, to form an insoluble fiber. This folding of an insoluble film is known as the Devaux (1935) effect, but it must not be supposed that this effect can be obtained with all films under all conditions. With B-films especially, the occurrence of the Devaux effect depends on a number of factors, such as the species of protein used (Goldacre, 1952),

previous treatment of protein (Kaplan, 1953) as well as the pH and composition of the sub-phase solution.

GENERAL CONSIDERATIONS OF THE PROBLEM

Keeping the preceding information in mind, I should like to suggest that some, and perhaps many, intracellular structures are formed by the surface-spreading of proteins as B-films at intracellular interfaces. This idea is certainly not new, having been suggested by Rideal (1945), Sobotka (1944), and especially by Danielli (1938) among others. But experimental attempts to apply this idea to the cell interior have been few. There are two reasons for this.

First, it is quite apparent that the information obtained from experiments on heterogeneous systems can be applied to the cell only by inference, or extrapolation. In other words, no direct proof or disproof of such an idea is possible as yet.

Second, earlier work on surface-spread enzyme protein seemed to show the disappointing fact that the biological activities of these molecules are lost upon surface-spreading.

Considering the first of these, it may be seen that, since one works with protein films and applies the findings to the cell, one faces charges of working with cell models, and that such an approach, providing no direct evidence for or against the idea, is a sterile one. However, if we reflect but a moment, we may see that a protein film or a fiber is no more and no less a model of the cell than is the test tube and aqueous enzyme solution of the biochemist. The tremendous accomplishments of biochemistry testify to the fact that, though the connection to the cell may be an ephemeral one, this is a priori no reason not to perform the experiment and to obtain the information. In line with this, it may be noted that biochemists in recent years have become more and more concerned with the state of organization and aggregation of enzymes in the cell (for example, Green, 1951; Hogeboom and Schneider, 1950; Rosenberg and Wilbrandt, 1952; Rothstein and Meier, 1948) as shown by the tremendous increase in the number of papers and reviews concerned with this phase. The emphasis is shifting to the localization of, and the association of, enzymes with cellular structures. Danielli (1951) goes so far as to say "... it seems advisable to regard all purified enzymes as at least partial artifacts until the contrary has been demonstrated."

The second reason for the dearth of work on the application of the protein surface work to the cell is the apparent loss of biological activity of the protein molecules concomitant with surface-spreading. In 1939, Rothen and Landsteiner gave clear evidence that the specific immunological reaction of antigen-antibody was retained with surface-spreading. But for the enzymes, Rothen (1947) was still able to say that the question of the retention of enzymatic activity by surface-spread enzyme was uncertain. If enzymes lost their activity with surface-spreading, there seemed little point in relating the surface-spreading of such proteins to the cell interior. I

shall enlarge upon this point in a moment, but here I should mention that the earlier work of Langmuir and Schaefer (1938), Harkins, Fourt and Fourt (1940), and Sobotka and Bloch (1941) gave clear indications that the indiscriminate application of such terms as "surface-denaturation" was unwarranted, and that a definite possibility existed that surface-spread enzymes do, in fact, retain their activity.

We may, therefore, ask ourselves the question, "What is the evidence from the cell that surface-spread protein forms the structures especially concerned in mitosis?" Examination of the many observations made on the cell membrane, the spindle figure, and chromosomes affords a great deal of evidence. The sum of such evidence, however, is at best only circumstantial, and I may warn that any one who can read and interpret these observations with his own ideas can build up an impressive case. Therefore, I shall merely mention a few pertinent observations.

The studies of the cell membrane by Davson and Danielli (1943) have led these workers to the conclusion that this structure is composed, at least in part, of protein molecules surface-spread at the cytoplasm-medium interface. Danielli (1951) and more recently Goldacre (1952) have used this concept as a basis for an explanation of cellular movement, as in ameboid movement, by the localized contraction of such a membrane. Polarized light studies of the cell membrane by Schmidt (1939) by Inoué and Dan (1951) and others, would support such a structural basis.

A comparison of the behavior of nucleoprotein fibers and of chromosomes toward the action of digestive enzymes by Mazia, Hayashi, and Yudowitch (1947) provided evidence pointing toward the fibrous configuration of these structures. Briefly, it was found that the chromosomes and the artificial fibers behaved similarly to the action of pepsin, trypsin, and papain.

Finally, in the case of the spindle figure, Inoué (1952) has demonstrated beautiful photographs showing the birefringence of this cellular structure. Similar studies have been made by Schmidt (1939) and Swann (1951). It is noteworthy that the intensity of this birefringence is constant in the metaphase figure. This is not what one would expect if the oriented constituents were found throughout the body of the spindle in equal concentration, for then the intensity of the birefringence would be greatest at the thickest portion of the spindle. One alternative possibility is that the oriented micelles are at the surface of the spindle, which would account for the uniform retardation found. Such an idea is supported by the cytological observations of many workers. For example, in the grasshopper spermatocyte, with the formation of the spindle figure the mitochondria become grouped in oriented fashion around the spindle but definitely excluded from the spindle. The interior of the spindle, apparently homogeneous, is in striking contrast to the cytoplasm surrounding it, and gives every appearance of being an immiscible phase in the surrounding cytoplasm. The question, so long a moot one in cytology, whether the spindle contains tangible fibers finds a rational answer in surface films of protein. For it is perfectly possible to fashion protein films in which the asymmetric molecules are oriented

and, therefore, exhibit birefringence, but with no sign of any visible fibers. However, with a slight alteration of conditions, striations in such a film can be produced, and in matter of fact, Kopac (1950) demonstrated this within the cell by his microinjection of an oil droplet and the development of the Devaux effect on the surface of this droplet. Finally, it may be noted in passing that the concept of lines of flow for the spindle figure (Pollister, 1941) is exhibited in protein films in the liquid phase. The techniques and measurements of Guastalla (1951) and Myers and Harkins (1937) illustrate that in such films the surface viscosity may be measured by utilizing the two-dimensional flow of asymmetric, oriented particles.

THE BIOLOGICAL ACTIVITY OF SURFACE-SPREAD PROTEINS

In our laboratory, then, the general approach has been to study the biological properties of surface-spread protein and to compare and relate these properties to the cell. To this end, we have made use of the Devaux effect as a means of rendering the protein films amenable to measurements of activity. The technique is simply to form a B-film of the protein to be studied at the surface of a Langmuir trough by spreading an excess of protein. The film may then be compressed by means of suitable movable barriers, and the solid fiber thus formed is, without further treatment, tested for biological activity.

Thus, with the enzyme catalase, Kaplan (1952a, b) has shown in our laboratory that the enzyme not only retains its biological activity with surface-spreading, but that the activity of the surface-spread enzyme resembles the action of the same enzyme within the cell. The experiments

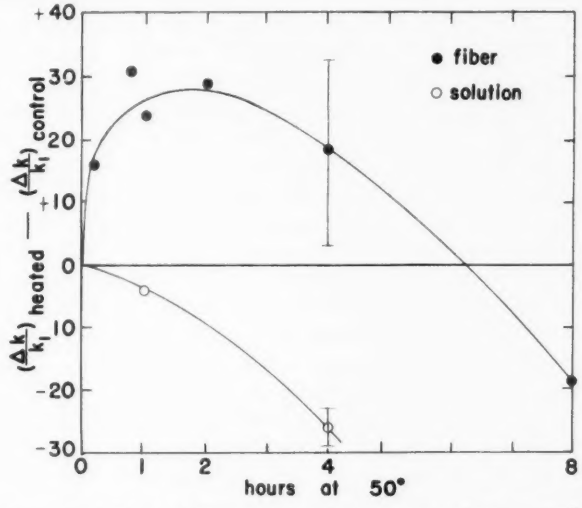


FIGURE 1. Activities of fibers and solutions maintained at 50° for varying times between runs; standard deviations are shown for the 4-hr. points. Each point is the average of four individual runs. Kaplan, *Jour. Coll. Sci.* 7: 382, 1952.

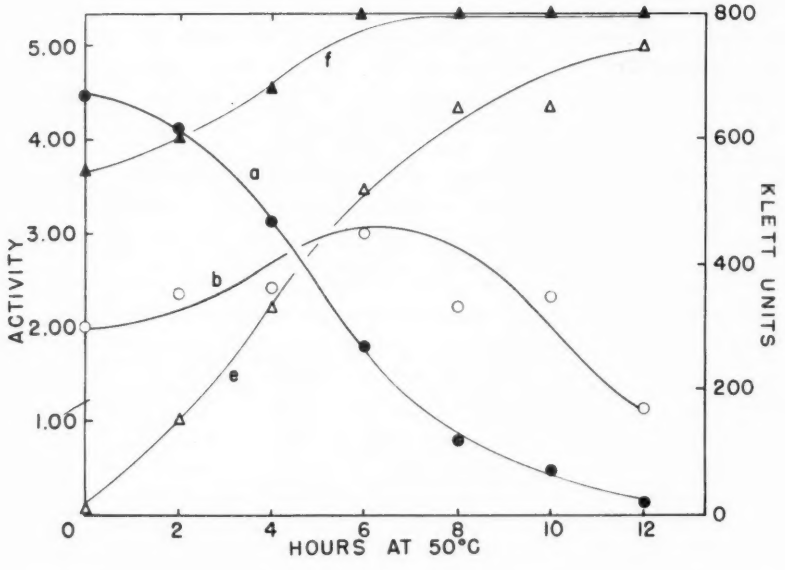


FIGURE 2. Curves *a* and *b* represent activities as follows: *a*, of hemolysate; *b*, of whole cell suspension. Curves *e* and *f* represent optical densities as follows: *e*, of supernatant of whole cell suspension; *f*, of centrifuged hemolysate. Kaplan, *Physiol. Zool.* XXV: 123, 1952.

were done as follows. A *solution* of crystalline catalase is subjected to the temperature range 50–60°C, the activity of the solution being tested periodically. It is found that the activity falls off regularly with time, as an effect of the elevated temperature. If now, however, the same enzyme is spread at the air-water interface and converted to a fiber, the temperature characteristic becomes radically changed. Instead of a regular inactivation of the enzyme, there is an initial activation of the enzyme as an effect of the temperature, followed by an inactivation (figure 1). This qualitative difference in temperature dependence indicates that the enzymic activity of the solid fiber is not due to contamination by globular protein. But more interesting are similar experiments done with red blood cells. The intact corpuscles show a strong catalase activity in suspension. When the effect of temperature is examined for such intact cells, the phenomenon of activation shown with the fibrous enzymes is also shown with the intact cells. If, however, the cells are hemolyzed and the enzyme leached out of the cell into solution, the catalase activity shows the same decrease in the same temperature range as the crystalline enzyme in solution (figure 2). In other words, the enzyme in the cell behaves as does the fibrous enzyme, the same enzyme removed from the cell behaves as the ordinary enzyme in solution.[1]

[1] Part of the increase in activity of the whole cell suspension is due to hemolysis, as pointed out by Kaplan. However, when this factor is corrected for, a temperature activation is clearly demonstrated.

These results with catalase we feel to be quite significant for the question of the relation of surface-spread protein and cell structure, but they have no bearing on the problem of mitosis. But some results obtained with proteolytic enzymes have a slightly greater bearing. The general method used in these experiments is illustrated in figure 3 (Mazia and Hayashi, 1953). A mixture of pepsin (enzyme) and egg albumin (substrate) is spread at the air-water interface and compressed to form a complex fiber of these two protein species. This fiber may now be removed from the trough and washed free of contaminating material. When placed in dilute HCl at pH 1.5 (figure 3, 6a), the fiber rapidly undergoes autodigestion.

Fibers of pepsin alone, or albumin alone, are completely inactive in the same digestion solution. The fact that a true proteolysis is occurring is shown by the accumulation of digestion products in the surrounding solution (figure 4). The figure shows, also, that the proteolytic reaction in the complex fiber is much more rapid than the action of the same enzyme, in comparable concentration, in solution upon albumin either as a fiber or in solution.

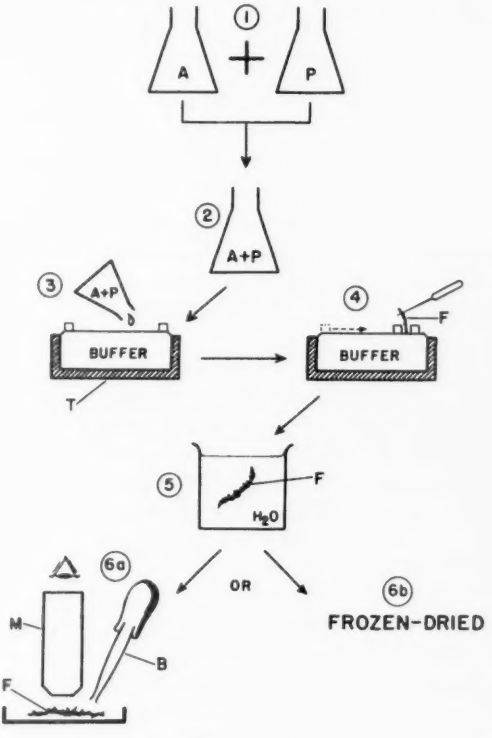


FIGURE 3. Diagram to illustrate the procedure in the formation and handling of pepsin-albumin complex films. Mazia and Hayashi, *Arch. Biochem. Biophys.* In press.

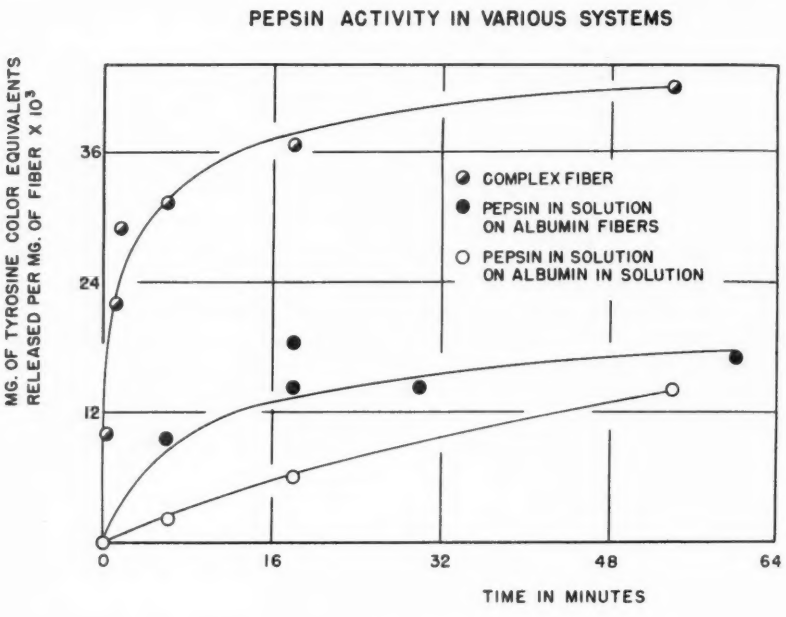


FIGURE 4. Pepsin activity in various systems. Mazia and Hayashi, *Arch. Biochem. Biophys.* In press.

Now the objection may be raised here that this autodigestion is due to the action of globular pepsin which somehow became adsorbed, or became incorporated as a contaminant in the fiber. The accumulated evidence of a number of different kinds of experiments, which I have not the time here to recount, points toward the view that this is not the case. The hydrolysis seems to be due to the action of surface-spread pepsin in the fiber. An interesting feature is that one pepsin molecule is capable of breaking down between 12–18 albumin molecules in this system.

The mechanism of this fibrous autodigestion is apparently the breakdown of a stabilized enzyme-substrate complex represented by the mixed fiber of pepsin and albumin (Hayashi and Edison, 1950). Evidence for this comes from the fact that if the pepsin and albumin solutions are placed at the interface separately, the fiber formed will not undergo autodigestion, but if the drops are allowed to mix before being placed on the trough, then the autodigestion proceeds rapidly. Likewise, as Table 1 shows, the pH at which the enzyme and substrate proteins are mixed affects the reactivity of the system, pH 4.4–6.6 forming active fibers, whereas at 7.2, inactive fibers are formed. From the experiments, it seemed that the pepsin and albumin reacted to form a complex in the mixture, and this was confirmed by titration of the mixture with saturated ammonium sulfate, and measuring the precipitate formed photometrically. Figure 5 depicts a smooth curve

TABLE I

OBSERVATIONS ON AUTODIGESTION. FACTORS INFLUENCING THE FORMATION OF AUTODIGESTIBLE FIBERS. NOTE ESPECIALLY EXP. 3, WHERE THE PH OF THE MIXTURE IS VARIED. THE SYMBOL + INDICATES AUTODIGESTION, – NO AUTODIGESTION.*

Exp.	Conditions					Fiber Formation		Autodigestion
	pH							
	Alb.	Pep.	Mixt.	Trough	Dig.			
1.	5.35	5.35		4.0	1.5	Alb. and pep. films separated on trough by threads, threads removed before compression.	Variation (a)	–
	5.35	5.35		4.0	1.5		Variation (b)	–
	5.35	5.35		4.0	1.5		Variation (c)	–
	5.35	5.35		4.0	1.5		Variation (d)	–
	5.35	5.35	5.35	4.0	1.5	Control. Standard fibers, mixing pepsin and albumin prior to spreading		+
2.	5.35	5.35		4.0	1.5	Alb. and pep. films placed dropwise separately on trough. Film then compressed.	Placed 30 cm apart	–
	5.35	5.35		4.0	1.5		Placed 10 cm apart	–
	5.35	5.35		4.0	1.5		Placed 2 cm apart	–
	5.35	5.35		4.0	1.5		Placed 0 cm apart	+
	5.35	5.35	5.35	4.0	1.5	Control. Standard fiber.		+
3.	4.4	4.4	4.4	4.0	1.5	Standard fiber with the mixture pH varied		+
	4.4	4.4	5.0	4.0	1.5	" " " " " " "		+
	4.4	4.4	6.0	4.0	1.5	" " " " " " "		+
	4.4	4.4	6.6	4.0	1.5	" " " " " " "		+
	4.4	4.4	7.2	4.0	1.5	" " " " " " "		–

*Hayashi and Edison, *Jour. Coll. Sci.* 5: 437, 1950.

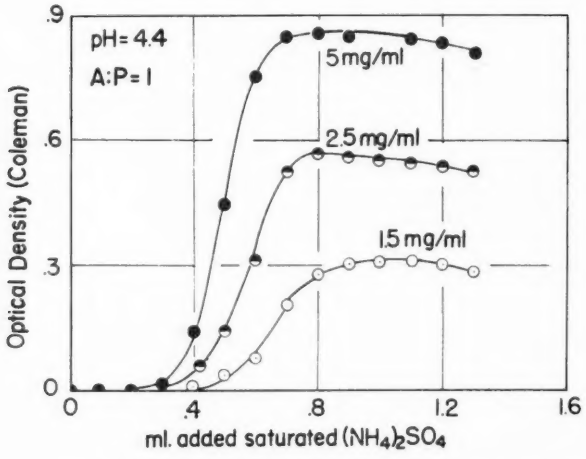


FIGURE 5. Precipitation curves for pepsin-albumin mixture at various total concentrations. The shape of the curves shows that the two protein species are being brought down simultaneously. Hayashi and Edison, *Jour. Coll. Sci.*, 5: 437, 1950.

of precipitate formation at pH 4.4, indicating that the two proteins in the mixture are being precipitated out of solution together. Pepsin in solution alone, or albumin alone, shows the same type of curve, the pepsin curve being practically superimposable on the complex curve, the albumin curve being displaced some distance to the right. This behavior of the pepsin and the albumin reacting together to the precipitating action of ammonium sulfate is shown throughout the range of pH 4.4–6.6 where, it may be recalled, autodigestible fibers are formed; but at pH 7.2, where the fiber is inactive, there is a sharp change in the titration picture (figure 6). The curves show the titration of pepsin alone, albumin alone, and the mixture of the two proteins. It may be seen that at this pH the two proteins precipitate out separately, indicating a failure to form a complex.

This picture of the action of surface-spread pepsin is not unique, for identical results may be obtained with trypsin, the only difference being the pH for autodigestion, which is 7.6 instead of 1.5. Likewise, the substrate may be varied, and other proteins used instead of albumin. Of interest is the fact that if nucleohistone be used as the substrate and pepsin as the enzyme, no autodigestion occurs. If, however, nucleohistone and trypsin are combined in a complex fiber, there is autodigestion at pH 7.6, not so rapid as in the pepsin case, but unmistakable, nevertheless (Hayashi, unpublished). The results pose the interesting possibility that chromosome changes in the mitotic cycle may be due to enzyme attack from within, rather than from without, the chromosome.

It is realized, of course, that to speak of the mitotic cycle in relation to the foregoing experiments and observations is pure speculation. The principal value in this discourse, however, is to bring to attention two im-

portant features: namely (a) surface-spread protein can retain biological activity and (b) surface-spreading of protein is a possible means of formation of cellular structures. These points are supported further if we consider cellular movements.

There are two general aspects of cellular movements in the mitotic process; namely, the movements of the chromosomes, and cytokinesis. For the chromosomes, Cornman (1944) has summarized the evidence in favor of the traction fiber as the contractile element drawing the chromosomes to the poles, and Inoué (1952) has demonstrated the birefringence changes of these fibers during cell division. For the division of the cytoplasm and associated changes in the cell, the studies of Marsland (1939) have led him to postulate the existence of a contractile portion of the cell surface in the equatorial region, which, in a sphincterlike contraction brings about the separation of cytoplasm. This mechanism bears a close similarity to that suggested by Goldacre (1952) for ameboid movement. While it is true that the mechanism of cytokinesis proposed by Dan (1943) differs sharply with that proposed by Marsland, and leaves no room for contractile elements, I feel reasonably certain that with more information a drawing together of these two mechanisms is possible. In any event, the mechanisms inherent in Marsland's idea and in the traction fiber hypothesis, in common with muscular movement, both call for the forcible shortening of elements, as against the alternative possibility of forcible elongation of elements (Dan, 1943).

One point which has received little attention in consideration of biological movement, but which I consider to be highly important, is that the con-

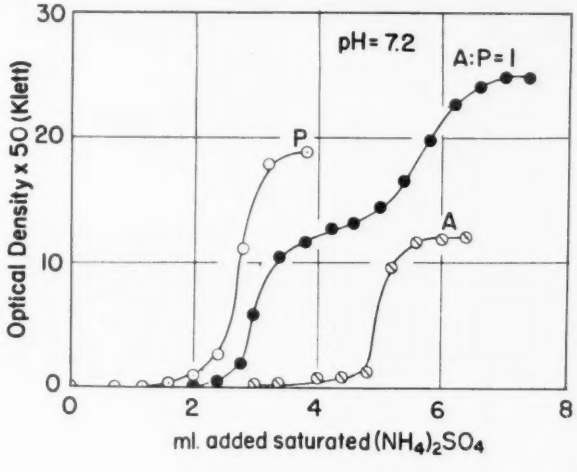
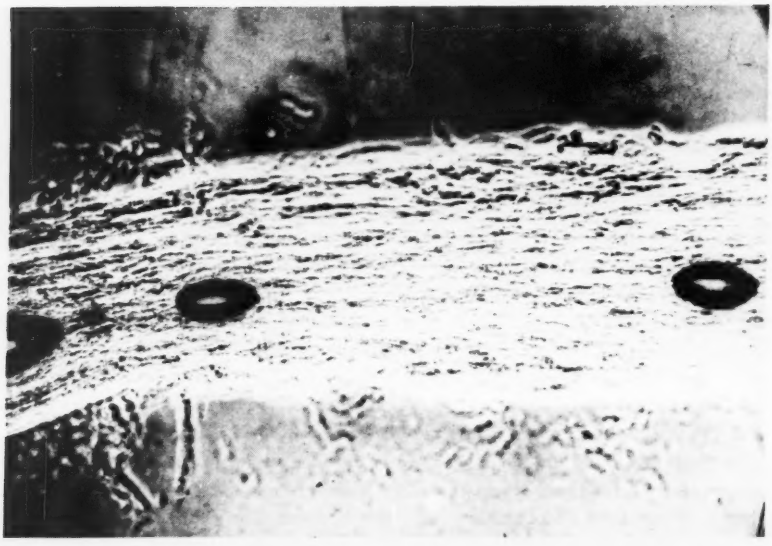
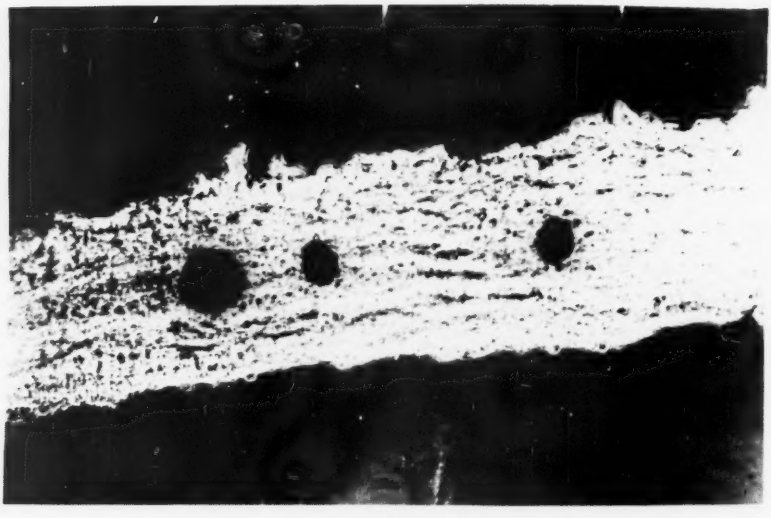


FIGURE 6. Precipitation curves at pH 7.2 for pepsin alone (P), albumin alone (A), and the pepsin-albumin mixture. The shape of the curve for the mixture shows the two protein species being brought down separately. Hayashi and Edison, *Jour. Coll. Sci.* 5: 437, 1950.



1a



1b

PLATE 1

Photomicrographs of surface-spread actomyosin fibers. Low power magnification. See text for explanation.

tractile elements require a structural continuity. If traction fibers, for example, by their contraction provide the basis for the movements of the chromosomes in anaphase, they must constitute a structurally continuous connection between the pole and the kinetochore of the metaphase chromosome, a connection which must be capable of overcoming the resistance of the sister chromatid pulling in the opposite direction and of pulling the anaphase chromosome to the pole. The distances involved are the order of tens of thousands of Angstrom units, and call for the intermolecular association of a number of unit molecules. Now, it is easy enough to postulate that the pole region, or perhaps the chromosome (Hughes-Schrader, 1924) sends out such a structurally continuous fiber to make the proper connections, but it is very difficult to visualize a physicochemical system capable of performing this apparently simple feat. A surface-spread protein system, however, not only provides the directional track for such a fiber, but also, insofar as I am aware, is the only physiologically-plausible system by which the formation of such a structurally continuous, contractile element has been demonstrated. The experiments on which this statement is based were done with the contractile protein of muscle, actomyosin.

Surface spread actomyosin, compressed to form a fiber, is shown in the upper photograph of Plate 1a. I should like to call your attention to the longitudinal striations of this fiber, which are probably folds in the accordion-pleated, two-dimensional film. Such folds are, however, actually closely apposed portions of the film, which, in their close association, have built up enough thickness to become visible, and under the phase contrast microscope give every appearance of being fibrillar elements. Kaplan (1953) has recently studied the factors affecting the formation of such folds, and has demonstrated that when such folds are not formed, continuity of structure does not exist.

When this fiber is immersed in a solution of ATP, a contraction takes place, and examination under high dry magnification shows that this contraction is apparently due to the kinking and knotting of the longitudinal fold-fibrils. The same fiber contracted is shown in the lower photograph, (Plate 1b), and one may note the change in position and shape of the three air bubbles, as well as the general change in appearance of the entire fiber. These fibers also show a strong birefringence positive with respect to the long axis, and some preliminary measurements by Dr. Inoué has indicated that with contraction there is a fall in birefringence, similar to that occurring in the traction fiber. These fibers also show a strong ATP-ase activity.

This contractile property of actomyosin with ATP was shown originally by Szent-Györgyi (1941). The fibers used by this worker, however, were prepared in quite a different manner, being precipitated upon ejection from a capillary (Weber, 1934). The lack of structural continuity in these fibers was shown by the fact that if the slightest tension were imposed on these fibers, the effect of ATP was to cause a specific elongation, rather than

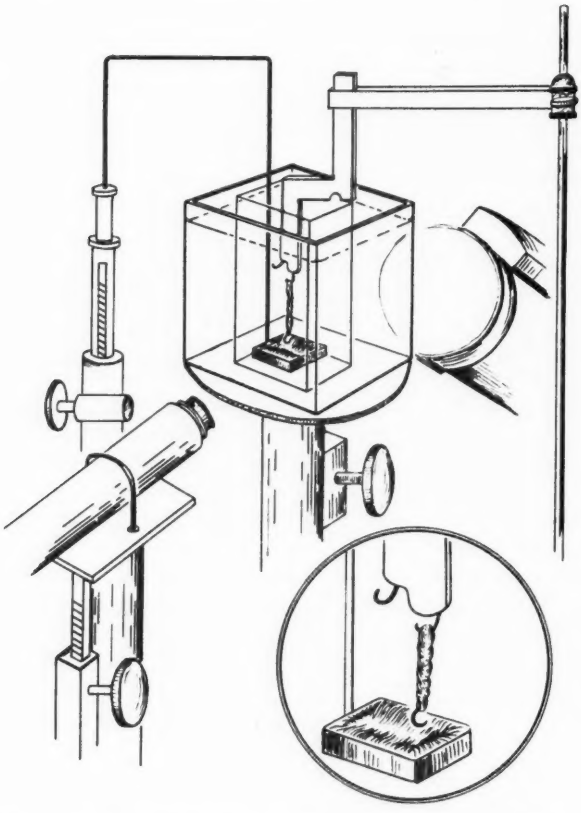


FIGURE 7. Apparatus for measuring isotonic contraction of fibers. Hayashi, *Jour. Gen. Physiol.* 36: 139, 1952.

a contraction. The individual molecules may have been contracting, but there was no intermolecular continuity.

The surface-spread fibers, on the other hand, demonstrate this structural continuity strikingly by their ability to lift a load, thereby performing mechanical work. Under the conditions of the experiment, the specific elongation effect of ATP is never shown by these fibers. The measurements were done by using the apparatus shown in figure 7. The fiber is supported by the stationary hook, and allowed to hang, loaded with a weight, freely in the reaction vessel. The movements of the weight are observed through a cathetometer telescope, the vernier scale which is capable of recording movements of .05 mm. Typical contraction curves with two different weights are shown in figure 8.

This isotonic contraction with ATP is readily reversible, so that a cycle of contractions and elongations may be demonstrated (figure 9). The con-

traction takes place in ATP with a salt concentration of 0.05 molar. If this salt concentration is increased to 0.25 molar and the ATP removed, elongation takes place, which may be stopped by lowering the salt concentration. If now ATP is re-applied, contraction occurs again.

The elongation of this protein fiber is not a forcible self-extension of the fiber, but rather a passive dragging out of the fiber by the fall of the suspended weight. This is shown in the following experiment (figure 10) where the loaded fiber is permitted to contract as before. The tension is now removed from the fiber by supporting the weight on a raised platform. The fiber in this condition is immersed in solution of higher salt concentration, where elongation can take place. If the elongation were a forcible self-extension, the fiber will become longer, the increased length being taken up by an additional looping of the fiber. After some time in this solution, the salt concentration is lowered again, where the fiber will maintain its new length. If now the weight is dropped, the fiber, straightened out, will show its new, increased length immediately. Examination of the results show that such is not the case. This point shows that the fiber has not elongated to any extent, but if the salt concentration is increased again, the imposed weight will now elongate the fiber rapidly. The elongation is a passive one.

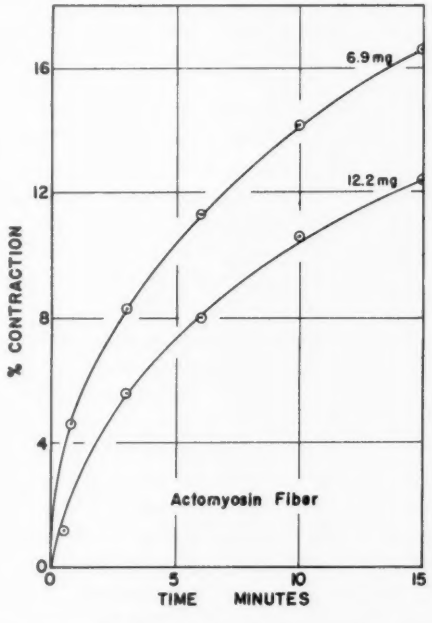


FIGURE 8. Contraction curves for loaded surface-spread actomyosin fibers. Two different weights shown. Hayashi, *Jour. Gen. Physiol.* 36: 139, 1952.

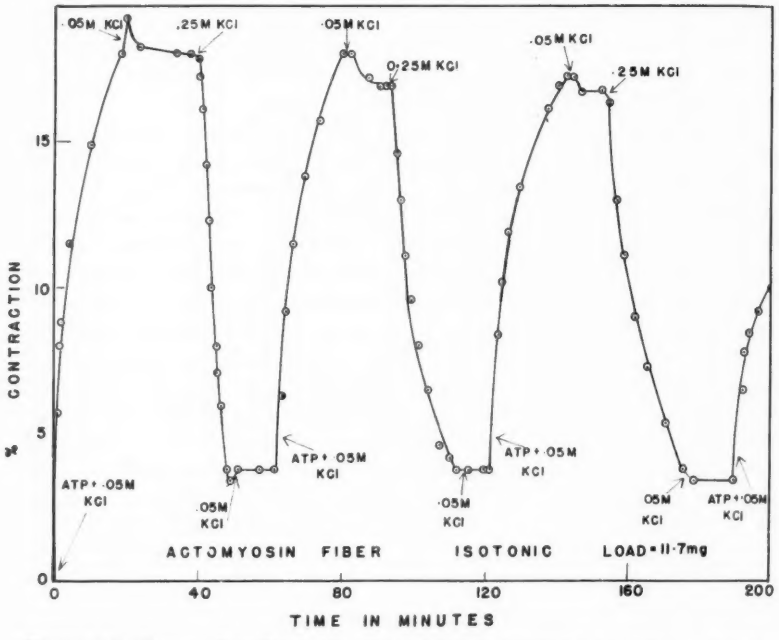


FIGURE 9. Contraction-elongation cycle of loaded actomyosin fibers. Hayashi and Rosenbluth, *Jour. Cell. Comp. Physiol.* 40: 495, 1952.

This result is mentioned to make the following point. The postulation of a forcible self-extension mechanism as a basis for biological movement, especially with a fibrillar system, is fraught with difficulties and calls for further assumptions of associated mechanisms. For example, if a fibril in the form of a folded polypeptide chain were capable of forcible elongation by an unfolding, its ability to push and to move a sizable resistance would be very small because of the fact that such a thread, of any length at all, would buckle in the process. It would be necessary to postulate a structural association with other, simultaneously elongating, elements into a geometric configuration suitable for such a pushing action.

The objection may be raised here that a contractile protein, such as actomyosin, while it may provide the basis for movement in a cell as highly differentiated as a muscle cell, would not be the basis for, nor indeed be found, in other types of cells. Evidence to the contrary, however, is provided by the work of Loewy (1952). This worker has extracted protein from the slime mold which has the same properties as does actomyosin.

Now, I must make a confession. We have made several attempts this summer to extract contractile proteins from dividing marine eggs, but with no success. This is, of course, disappointing, but it does not mean thereby that the proteins are not present. It may mean only that in such non-specialized cells, the amount of contractile material is so small that one must

work with much larger amounts of starting material. Or again, it may mean that the contractile proteins of such material have different properties so that the extracting medium for muscle actomyosin is not suitable for such proteins. Additional reassurance is provided by the cytological observations of Hughes-Schrader (1924), who demonstrated that the contractile fiber may originate in the chromosome. We have found that surface-spread nucleoprotein fibers have contractile properties. If such a fiber is immersed in a buffer at pH 2.0, there is a rapid contraction and condensation of the fiber. Even more exciting are the results of the experiments of the Russian investigator Zbarskii and his co-workers (Zbarskii and Perevoshchikova, 1951 a, b) who have found that precipitated fibers of nucleoprotein will contract specifically in a reaction with ATP. The reaction is dependent on the association of the histone fraction with the residual acid protein fraction, either fraction alone showing no contractile properties. We are, therefore, planning an investigation of these proteins in the surface-spread condition in the near future.

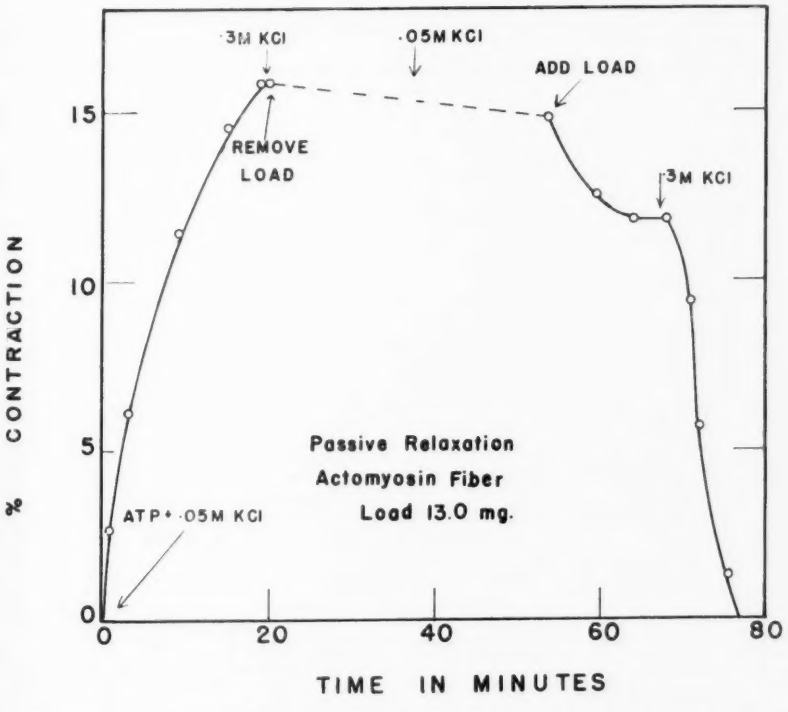


FIGURE 10. Passive elongation of actomyosin fiber. Point to be noted is marked "Add load"$_1$. Here, loaded fiber has been allowed to straighten to full length after period of "relaxation," but there is practically no increase in length. Subsequent elongation is due to dragging out of fiber by load imposed. Hayashi and Rosenbluth, *Jour. Cell. Comp. Physiol.* 40: 495, 1952.

It is readily seen that all of this discussion is based on a general assumption; to wit, that some cellular structures are formed of protein surface-spread at intracellular interfaces (Hayashi, 1951). Admittedly, the application of information gleaned from the study of surface-spread protein to problems of cell division is highly speculative, but I hasten to point out that this speculative tone is exaggerated because of the newness of the work on the biological properties of proteins in such a physical state. And it is a fact (we take comfort in this) that of the several proteins that we have studied along these lines, each of them has shown properties strikingly analogous to the protein in the cell. To say that this supports the assumption is as yet presumptuous, but it at least encourages one to continue the work.

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Vol. LXXXVII, No. 835 The American Naturalist July-August, 1953

THE ABILITY OF INSECTS TO DISTINGUISH NUMBER*

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A series of new discoveries has thrown some light on the perplexing life and behavior of social insects. The classic research of Karl von Frisch and his co-workers on "the dances of the bees" revealed the existence of a well-developed system of communication among insects. By performing certain circling dances which are accompanied by definite body movements, a bee is able to communicate information about the location and type of nectar plants.

Besides these orientation dances, the writer of this article noticed some more types of "alarm" and "warning" dances, and a strange phenomenon called "bees' struggle" (Leppik, 1951a, 1951b; Bolle, 1952). Further contributions to the problem of bee "dances" have been made by F. Schneider (1949), P. Tschumi (1950), in Switzerland, M. H. Haydak (1932), V. G. Milum (1947), and A. L. Kroeber (1952) in the United States, Hein (1950) in the Netherlands, and several other workers in biology.

These experiences of several workers have now enabled us to decipher the main part of a "sign language of bees"[1] and to assume the existence of further communication systems of social insects.

The next question that arose was: can insects distinguish and memorize numbers, and if so, in what manner?

Many flowering plants, whose fertilization and seed production is dependent upon insect pollination, have flowers with constant numbers of petals, sepals, and other floral parts. The dissimilarity of trimerous, tetramerous, and pentamerous flowers is so striking as to be able to serve as possible pointers for the pollinating insects. The amazing behavior of these insects

*Data for this paper were recorded while the author was in the employment of the State Horticultural Research Institute and professor at the Technical University of Munich, Bavaria.

[1] The term *"language of bees"* is criticized for its anthropomorphic meaning which is misleading if applied in the case of communication of insects.

The word *"language"* pertains to the tongue (lingua), which is the main organ used in human verbal speech. But insects do not use the tongue in their communication. Kroeber (1952), discussing different viewpoints, concludes that "language" is not the appropriate term to express the way of communication of insects.

Von Frisch uses in German the term "die Sprache," in quotation marks which has, however, a different origin and a more general meaning than our "language." "Sprechen" means "to speak" in general and does not pertain to the tongue.

The author of this article proposed the introduction of a new term *"melittolexis"* (or "apilexis"). Melitta in Greek (μέλιττα) means bee and lexis (γλέξις, from λεγω) means "mode of expression," the compilation of signs or symbols (as a lexicon), and a "language" as a general concept. Dialect (διαλεκτος) is a local variety of a general language.

in remaining *steadfast*[2] to certain flower species, indicates that they have considerable ability of orientation among them. The steadfastness of bees, bumblebees, butterflies, and moths was established by several flower ecologists (Lovell, 1918; Kugler, 1943; Werth, 1949; Frisch, 1950) and confirmed by the writer experimentally (Leppik, 1951). H. Kugler also recently observed some groups of flies (Eristalomyia and Syrphus) remaining steadfast to certain flowers.

From these circumstances it seems quite plausible to assume that some groups of pollinating insects have developed a certain ability to discriminate number. The writer (1948) attempted to bring some light to this question by special experiments and field observations.

A series of tests have been performed in the State Horticultural College and Research Institute at Weihenstephan in Bavaria during the years 1945-1950. This institution with its several research departments and sections dealing with flowers and ornamental plants, surrounded by rich wild flora, offered a most heterogenous material for these types of experiments and observations. The main results of this study have been discussed previously (Leppik, 1948a, b; Bolle, 1952); whereas the manner in which the insects have been found to distinguish number is to be delineated briefly in this article.

EXPERIMENTS WITH MARKED BEES

Two closely related species of Mexican marigold, *Tagetes patulus L.* and *T. signatus Bartl.*, were used for the experiments with marked bees (fig. 2). These species differ in the number of ray flowers. *T. patulus* normally has several ray flowers in contrast to *T. signatus*, which possesses regularly only 5 petaloid rays. Honey-bees, wild bees and other pollinating insects distinguish between these species. It could be assumed therefore that *T. signatus* is a product of a long selective activity of some specialized pollinators, which have preferred the pentamerous forms among *T. patulus*. This assumption is supported by the fact that *T. patulus* has a clear tendency toward polymorphism since pentamerous and hexamerous types are not rare among the garden forms of this species.

Beside having different numbers of ray flowers *T. patulus* and *T. signatus* can be distinguished further by their different odors, color and size of heads.

In a bee colony adjusted to certain marigold forms, some ray flowers were removed from a number of heads. In this way the number of rays could be changed from 10 to 5, from 5 to 2, etc. (table 1). The majority of the marked bees immediately avoided the abnormal heads. Others became confused by the different numbers in ray flowers and refused part of the abnormal blossoms,

[2] *Steadfast* is recommended by H. M. Blegen to express the attitude of pollinating insects in visiting the flowers of only one type at a time and in not mixing the nectar or pollen of different plant species. It was believed formerly that bees are individually *constant* to some single or few species, which faithfulness of bees was called *"flower fidelity"* (J. H. Lovell, 1918). This viewpoint is not quite correct and steadfastness is in our case not a synonym for "constancy." In German the term "Artstetigkeit" is used since long instead of "constancy."

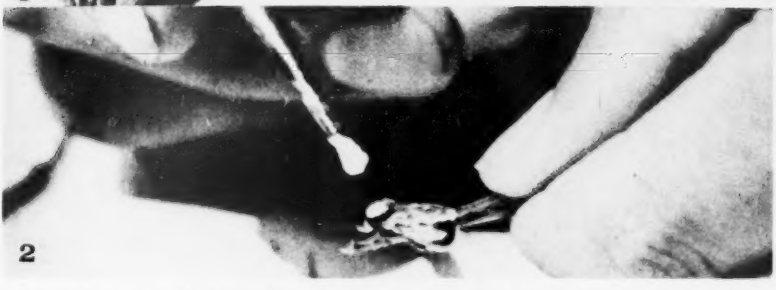

FIGURE 1. A moth (Macroglossa) observes the characteristics of a flower *(Verbena aubletia)* from short distance before stretching its proboscis into flower tube.
FIGURE 2. A bee is marked with colored spot on back for observations.

TABLE 1

RECORDS OF FLOWER VISITS OF BEES REGISTERED ON A FLOWER BED BEFORE AND AFTER CHANGE IN THE NUMBER OF FLOWER PARTS OF *Tagetes patulus* AND *Tagetes signatus.*[1]

	Number of ray flowers	Length of Observation[2]			
		1 hr.	2 hr.	3 hr.	4 hr.
Tagetes patulus Normal	Many	78	73	75	71
T. patulus ray flowers Reduced to 5	5	3	8	16	23
T. patulus ray flowers Removed	0	....	....	....	....
Tagetes signatus Normal	5	52	53	46	47
T. signatus ray flowers Reduced to 2	2	4	2	11	31
T. signatus ray flowers Removed	0	....	6	3	5

[1]The flower bed contained during the experiment 1211 flowers of *T. patulus* and 7831 blossoms of *T. signatus.*

[2]During the first hour of observation, all blossoms were normal. After 1 hour, the ray flowers in 100 blossoms were reduced or removed. Further observations were made during 2, 3, and 4 hours. In the beginning most bees refused the abnormal flowers, but started to visit them again later.

or visited both types. Scout-bees, as much as could be observed, did not pay any attention to this change. If a great number of heads were changed in this way, bees finally adjusted themselves to the new combination. In the case of intense odors, bees decided on the basis of scent.

According to these experiments, bees are able to remember and distinguish the numbers 1, 2, 3, 4, 5, 6, 8, 10, and 12. It is remarkable that the numerical system of bees does not contain the "magic" numbers 7, 9, and 13 but have double meanings for 3 and 5. The most favored number for bees is 5.

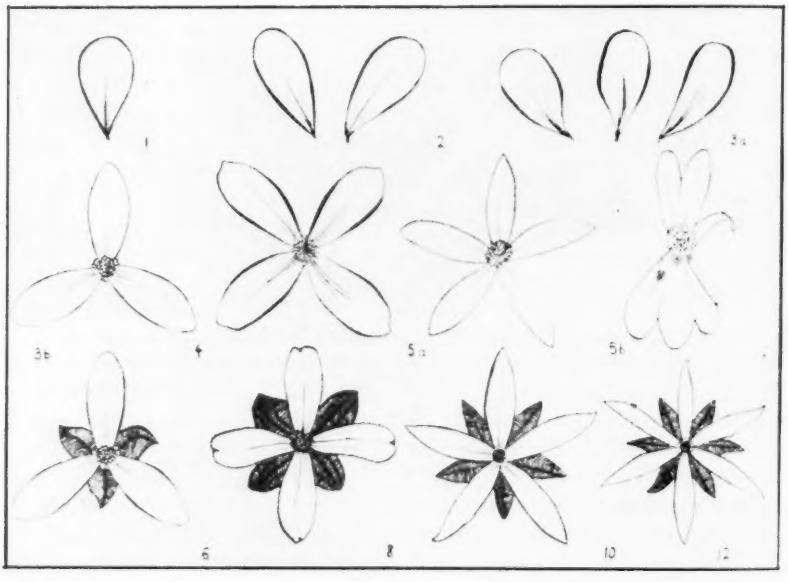

FIGURE 3. Symbols for numbers 1–12, which a worker bee was able to memorize and distinguish if these were connected with flower types.

The way a bee uses numbers is quite different from our way. A bee cannot "count" but must have the numbers expressed in figures that it can easily memorize. The bees are able to distinguish only some few objects one from another. They were unable to make a clear distinction between marigold heads with 3 and 4 petals and these numbers seem to be the limit of their "calculative" ability. They can, however, easily remember higher numbers if these are expressed in symmetrical figures. Figure 3 contains the symbols and figures for numbers a bee is able to distinguish; fig. 4 shows how these numbers are expressed in flower types.

SOME RESULTS FROM THE FIELD OBSERVATIONS

Table 2 is a condensation of numerous observations, made on about 500 wild and garden plants and their pollinators. Most specimens of table 2 il-

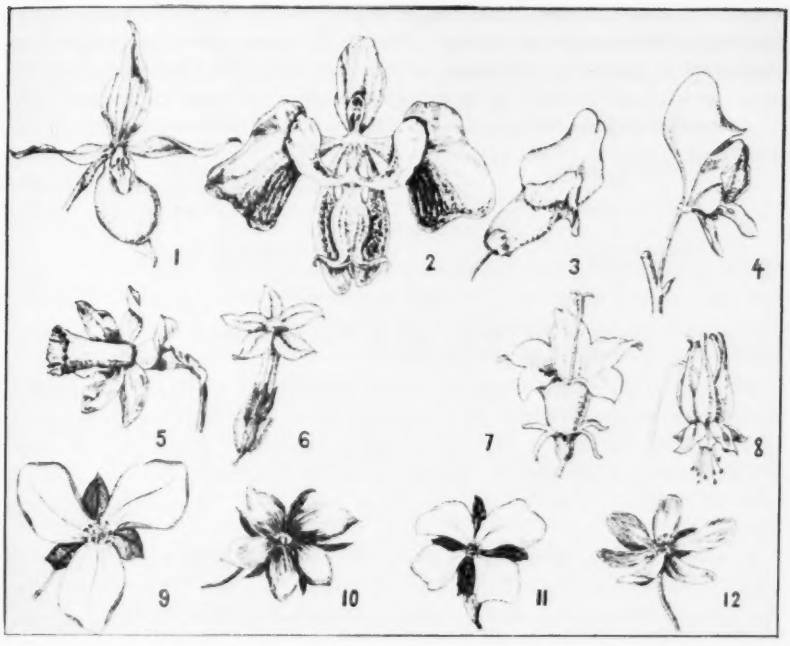

FIGURE 4. The combination of numbers, symmetry and colors expressed in flower types. First row (1, 2, 3, 4) are zygomorphic, second row (5, 6, 7, 8) protected and third row (9, 10, 11, 12) radiate types. (Redrawn by G. Homburger.)

lustrate some ecologically standardized communities, where the relations between flower types and pollinators are well established. Some further examples are taken from the extensive flower gardens of the Weihenstephan Horticultural College. In the last case several hundreds of kinds of flowers were permanently exposed to the insect visitors. By these circumstances every pollinator could make its own choice among the flower types it pre-

TABLE 2

TOTAL NUMBER OF POLLINATORS REGISTERED DURING FIELD OBSERVATIONS IN 1948–1949 ON ABOUT 500 SELECTED SPECIES AND VARIETIES OF WILD AND GARDEN FLOWERS

Flower	Beetles, Weevils	Bees	Butterflies	Moths	Bumble-bees
Zygomorphic types, mainly bumble-bee flowers	(165)	(22)	....	....	2756
Protected types with definite number of flower parts	....	....	1375	1261	....
Radiate types with definite numbers	140	2761	....	....	(261)
Simple types with indefinite numbers	1876	18	....	....	(63)

ferred. These selected examples of flowers are arranged in table 2, according to their evolutionary trend from simple →, radiate →, protected →, to zygomorphic types,[3] as follows:

Zygomorphic types	Definite number of flower parts and bilateral symmetry
↑	
Protected types	Definite number of flower parts and protected nectaries
↑	
Radiate types	Definite number of flower parts
↑	
Simplex types	Indefinite number of flower parts

The main groups of pollinators are placed corresponding to their specialization and skill from left to right.

By such arrangement of material we can establish a definite parallelism between the numbers expressed in flower types and the ability of main groups of pollinating insects to distinguish these numbers. The appearance and differentiation of numbers in the phylogeny of flowering plants can be coordinated with the gradual development of the number discrimination of pollinating insects.

It is remarkable, however, that the bumble-bees preferred zygomorphic types instead of numerical symmetry of radiate flowers. When the bumble-bee flowers are absent, these highly specialized pollinators visit radiate, capitulate and simple types,[3] or manage even nectar robbery, from flowers not readily accessible to them.

DISCUSSION OF RESULTS

Both experiments with marked bees (fig. 2) and field observations (fig. 1) ascertained the ability of pollinating insects to distinguish definite flower types. But this ability, which seems to be dependent upon the stage of psychic development of the insects, varied considerably among the groups studied. Flies, beetle, and weevils commonly did not distinguish more than a few objects one from the other. These insects did not pay any attention to the change of numbers in flower parts. In the event they had to choose, beetles preferred commonly the *simplex*[3] type with unrestricted number of petals and stamens.

Bees, on the contrary, were able to distinguish and memorize different combinations of numbers as arranged in fig. 3. They also reacted instantaneously to change in the numbers of flower parts.

In addition to establishing the bees' knowledge of numbers, our experiments showed the presence of a keen sense of symmetry among the pollinating insects, which is especially well developed among bumble-bees.

[3] Some main flower types are indicated according to the classification of the writer (1948, 1953 and fig. 4); *Simplices*, with unrestricted number of petals, and stamens are one of the oldest. *Radiatae* and *protectae* (nectar deposits protected) contain a definite number of petals and sepals. *Zygomorphic* types are the youngest.

Numerous flower types mirror the high level of sense perception among the higher pollinating insects. Besides their colors, symmetry, odors, and number of flower parts, bees are able to remember and distinguish the nectar plants from numerous flowers exposed to pollinators at the same time. Every pollinator remembers the main characteristics of its flowers, knows where the nectar deposits are hidden, and is familiar with the occasionally complicated mechanism of pollination.

SOME CONCLUSIONS

One of the most important conclusions which can be derived from the experiments mentioned above is that pollinating insects show a strong tendency to consider a flower type as a whole. Pollinators are attracted to the flowers neither by their colors, nor by odor alone, as was assumed by several earlier ecologists. If a pollinating insect is *steadfast* to certain flower type, it must remember all essential characteristics of this flower: size, color, number of flower parts, symmetry, and odor. Even the speediest pollinators, such as sphinx moths, before touching the plant, take time to examine the main characteristics of every flower (fig. 1). Removal of only a single petal or exposure of differently colored varieties of the same species causes bees to notice the difference, but they consider other characteristics of this flower and will continue to visit all varieties as long as the nectaries are not removed.

The study of flower types in connection with pollinating insects may reveal some principal factors in the evolution of flowering plants. The ability of pollinating insects to distinguish and remember numbers may have an important selective value among different varieties and ecotypes of flowering plants. In this way, pollinating insects can direct the evolution of flowers toward certain types with definite numbers of flower parts, as is the case of many flowers such as radiate and protective types (fig. 4, middle row).

SUMMARY

Pollinating insects, bees, bumble-bees, butterflies, and moths have been found able to distinguish numbers, if these are expressed in symmetrical figures as pictured in fig. 3. This ability seems to be dependent upon the stage of psychic development of insects and varies considerably among different groups.

Different groups of numbers are clearly expressed in the flower types (fig. 4) and may serve as pointers for pollinating insects to find their plants. The comparative study of trimerous, tetramerous, and pentamerous flower types provides a new approach to the evolution of flowering plants.

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Vol. LXXXVII, No. 835 The American Naturalist July-August, 1953

MULTIPLE SEX CHROMOSOME MECHANISMS IN THE GRASSHOPPER GENUS PARATYLOTROPIDIA

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It was shown by King and Beams (1938) that a rare species of grasshopper, *Paratylotropidia brunneri* Scudder, possessed a type of multiple sex chromosome mechanism which may be formally designated as X_1X_2Y (male) and $X_1X_1X_2X_2$ (female). X_1 and Y are metacentric elements, while X_2 is an acrocentric. This peculiar mechanism resembles that found in one section of the Mantoidea (White, 1941; Hughes-Schrader, 1950), but differs from it in a number of ways. King and Beams postulated that the sex chromosome system found in *P. brunneri* had arisen in evolution as a result of two chromosomal fusions, one between an originally acrocentric X and an autosome which we may call A and a second between the unfused homolog of A and a second autosome, B. Thus X_1 came to be composed of the original X and A, a neo-Y (confined to the male line) was formed out of A and B, while the unfused B chromosome became X_2. The pairing of the three elements during the meiosis of the male seems to support this hypothesis, since the "right" limb of X_1 pairs with the "left" limb of the Y (these two limbs representing the "A" pair of autosomes); while X_2 pairs with the "right" limb of the Y (these representing the "B" autosomal pair).

The relative lengths of these various chromosomal arms, together with the distribution of heterochromatic segments in them, showed, however, that other changes (in addition to the two postulated fusions) had occurred in the transformation of an XO into an X_1X_2Y mechanism. It accordingly seemed very desirable that a cytological study should be made of the two other species of the genus *Paratylotropidia, P. beutenmulleri* Morse and *P. morsei* Rehn and Rehn. The present paper reports such an investigation and attempts to trace the evolutionary history of the sex-determining mechanism in this genus of grasshoppers.

DISTRIBUTION OF THE SPECIES

The taxonomy and distribution of the three species of *Paratylotropidia* has been dealt with by Rehn and Rehn (1943). *Brunneri* occurs from South Dakota, through Iowa, western Illinois, Kansas, Missouri and Arkansas to south-central Oklahoma and (possibly) north Texas; in addition it has recently been found near Nashville, Tennessee (J. J. Friauf, *in litt.*). *Beutenmulleri* is confined to the southern Appalachian mountains of Virginia, North Carolina and South Carolina; while *morsei* is restricted, as far as is known, to a small area of the Ouachita mountains of western Arkansas and eastern Oklahoma. The range of *beutenmulleri* is thus separated by a gap of some hundreds of miles from those of the other two species; while the

range of *brunneri* seems to almost encircle that of the very local endemic species *morsei*. There seems to be a good possibility that *brunneri* and *morsei* may be sympatric in some areas of Oklahoma and Arkansas, but they have not yet been actually found to occur together at any locality.

All three species are extremely rare, local forms which seem to be characteristic of oak-hickory woods, where they hop about clumsily among the dead leaves. Morphologically, *brunneri* and *morsei* are very similar (although clearly distinct species, on the evidence of the male genitalia, shape of tegmina and other features); while *beutenmulleri* is considerably different from the two 'western' forms.

During the summer of 1952 with the help of Nicholas J. White and L. Herbert Bruneau we collected all three species of the genus at the following localities:

Beutenmulleri—three miles S. of Caesar's Head, Greenville Co., S. C., June 29.
Morsei—five miles NE. of Finley, Pushmataha Co., Okla., July 19–20 and Aug. 12.
Brunneri—Madill, Marshall Co., Okla., July 20.

The material of *P. brunneri* consisted only of two females, which were not studied cytologically; we consequently rely on King and Beams' account of the chromosomes of this species. Testes of three males of *beutenmulleri* and one of *morsei* were fixed in Navashin's fluid, sectioned at 24 micra and stained in gentian violet by Newton's method. It would have been desirable to make aceto-orcein squash preparations as well in order to facilitate study of pachytene nuclei, but circumstances prevented the collection of more male individuals for this purpose.

OBSERVATIONS ON *P. BEUTENMULLERI*

Spermatogonial metaphases of this species (fig. 1a) show 22 chromosomes, of which one is a large metacentric, whose arms are only slightly unequal in length. The "short arms" of the 21 acrocentrics are clearly visible and all seem to be about the same size.

First metaphases (fig. 1e) show ten autosomal bivalents of which from 2 to 4 possess both a proximal and a distal chiasma, while the remaining 6 to 8 have only a distal one. There is also a large sex bivalent including the metacentric element already mentioned, the longer limb of which is paired with an acrocentric chromosome. *P. beutenmulleri* is accordingly a species in which a single fusion between the X and an autosome has given rise to a neo-X : neo-Y mechanism in the male. The large metacentric is the neo-X, of which the shorter limb (here designated XL) represents the original X, while the longer one (XR) was formerly an autosome. At first metaphase XR appears to be almost exactly the same length as the Y; the association between these two is strictly terminal at this stage—in fact their ends usually appear as if slightly separated or connected by a faint thread.

From such first metaphase stages one might conclude that XR and Y (which have undoubtedly been derived from the two members of a pair of autosomes) are still homologous throughout their entire length. A study of

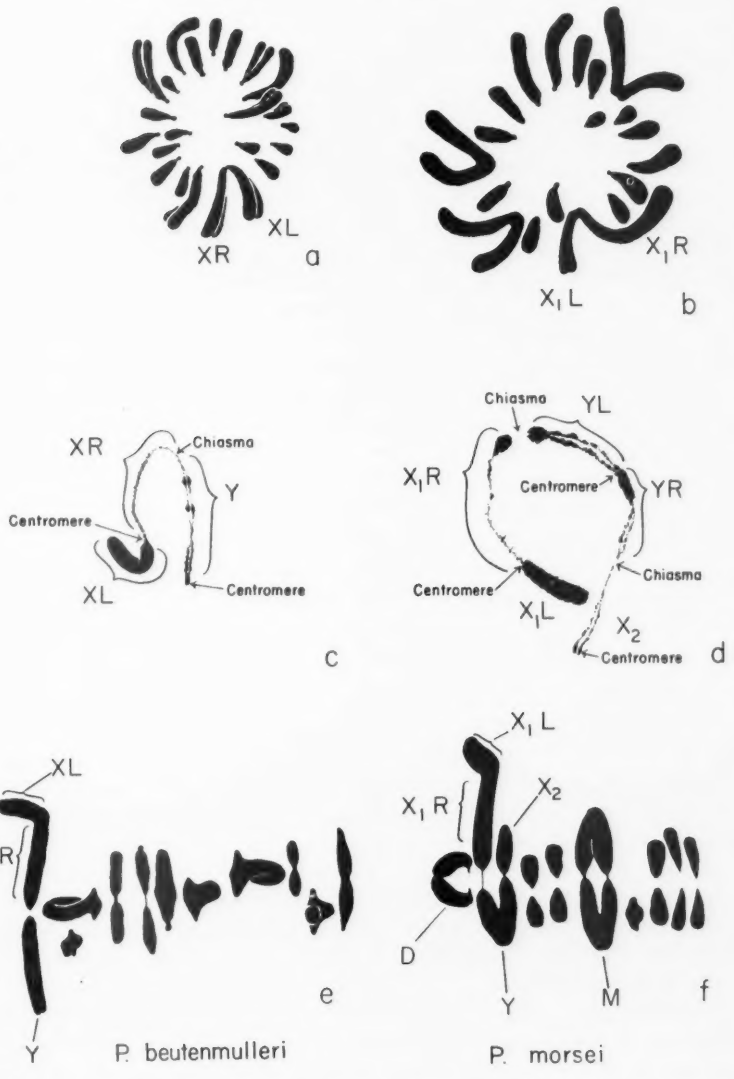

FIGURE 1. a—spermatogonial metaphase of *P. beutenmulleri;* b—the same of *P. morsei;* c—the sex bivalent of *P. beutenmulleri* at diplotene; d—the sex trivalent of *P. morsei* at diplotene; e—first metaphase of *P. beutenmulleri* (side view); f—first metaphase of *P. morsei.* M, the large metacentric bivalent; D, the bivalent which forms a chiasma in the short arm.

pachytene and diplotene stages, however, shows that this is not so (fig. 1c). The heteropycnotic regions do not show up during the prophases of meiosis as clearly as they do in some other species of Melanopline grasshoppers, but one can see that there are three blocks of condensed heterochromatin in the Y (a long one at the proximal end and two shorter interstitial ones), whereas in the corresponding regions of XR there are no such deeply-stained condensed segments. Even in early diplotene the association between XR and the Y is an effectively terminal one, there being no sign of an interstitial chiasma. Thus if the association is due (as we are inclined to believe) to a terminal chiasma, this chiasma must be formed very close to the end and probably undergoes complete terminalization almost immediately. The distal ends of XR and the Y are apparently euchromatic, and there is presumably a short "pairing segment" at the end of each. XL always shows up very clearly at diplotene, being very thick and condensed. There seems to be no possibility of determining whether the centromere of the metacentric X was derived from the original X or from the autosome which fused with it.

We have observed no cases of failure of pairing between the neo-X and the neo-Y, either at diplotene-diakinesis or at first metaphase. Nor have we seen any instances of mal-orientation at first metaphase or anaphase. The sex chromosome mechanism of *P. beutenmulleri* consequently appears to be highly efficient, in spite of the fact that its functioning seems to depend on pairing of minute segments at the distal ends of XR and Y, and the subsequent formation of a chiasma between them.

OBSERVATIONS ON *P. MORSEI*

Spermatogonial metaphases of *morsei* (fig. 1b) show 19 chromosomes, of which four are large metacentrics, the remaining 15 being acrocentric. Of the four metacentrics, one has the two arms markedly unequal, while in the case of the other three the inequality is rather slight. This karyotype is essentially identical with that reported by King and Beams for *P. brunneri*.

First metaphases of *morsei* show one very large sex-trivalent and 8 autosomal bivalents. One of the latter is very large and is composed of a pair of metacentric chromosomes. Almost invariably it forms a large ring at first metaphase, there being presumably a terminalized chiasma in each arm. In a single cell, out of several hundred observed, the two chromosomes composing this bivalent were only associated on one side of the centromere, the chiasma which is normally present on the other side having apparently failed to form. The second bivalent, in order of size, is remarkable because in spite of being acrocentric it usually forms a chiasma in the minute "second arm" as well as in the main limb. It consequently appears as a D-shaped structure in side views of first metaphase. Two other medium-sized bivalents are ring-shaped at first metaphase in some cells but if so, both chiasmata are in the main limb. The remaining four bivalents seem to invariably form a single chiasma each, in a distal position.

The sex trivalent is of the same form as in *P. brunneri*, described by King and Beams, that is, it consists of a metacentric X_1 and an acrocentric X_2, paired with the two limbs of a metacentric neo-Y (fig. 1f). Of the five chromosome limbs in the sex-trivalent (that is, not counting the minute "second arm" of X_2, which is not normally visible), X_1R is by far the longest. YR is the second longest, while X_1L, YL and X_2 are shorter and all about the same length. X_1L clearly represents the original X, that is, it corresponds to the XL of *P. beutenmulleri*. Although, as already stated, it is about the same length as X_2 it seems to be invariably thicker and consequently more massive than the relatively slender X_2.

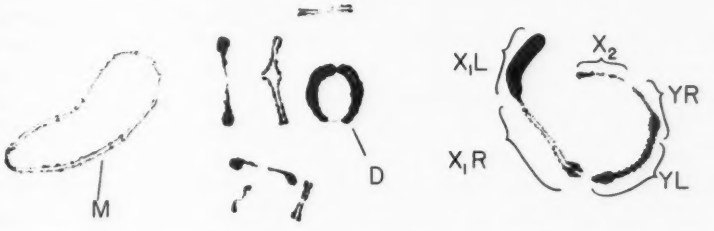

FIGURE 2. The sex-bivalent and the eight autosomal bivalents from an early diplotene nucleus of *P. morsei*. Lettering and symbols as in fig. 1.

First anaphase results in X_1 and X_2 passing to one pole and the neo-Y to the other. Consequently two classes of secondary spermatocytes are formed, with 10 and 9 chromosomes respectively. Both categories of second metaphases contain two large metacentric elements, but in those with ten chrososo mes these are X_1 and the large autosome, while in those with nine they are the Y and the large autosome. The X_1 is clearly distinguishable from the Y in second divisions because its limbs are more markedly unequal.

In order to analyze the structure of the sex-trivalent in greater detail it is necessary to examine early diplotene nuclei (fig. 1d and fig. 2). At this stage X_1L shows strong positive heteropycnosis and usually appears as a great sausage-shaped mass which stains intensely; in some cells it shows signs of a longitudinal split. X_1R is mostly euchromatic in appearance but its distal end consists of a conspicuous heterochromatic block. There is a similar sized block of heterochromatin at the distal end of YL. It certainly looks as if these two blocks represented pairing segments; at the earliest stage of diplotene they are terminally attached, apparently separated by a non-staining gap. Presumably there is a terminalized chiasma between them, but we have never been able to see this chiasma in an interstitial position. There are some indications that the conspicuous blocks of heterochromatin near the ends of X_1R and YL have a minute euchromatic region distal to them. If so the chiasma may be formed in this region rather than in the heterochromatin.

We cannot directly observe the centromere in the Y-chromosome at diplotene. Most of YL consists of a series of heterochromatic blocks; and if the relative lengths of the arms do not change greatly between diplotene and metaphase the proximal part of YR is also strongly heteropycnotic. The distal part of YR, however, is apparently largely euchromatic, possibly with a few minute heterochromatic sections in it. X_2 is likewise largely composed of euchromatin, with some tendency to be more condensed at its proximal (centromeric) end. The association between X_2 and YR is a terminal one, just like that between X_1R and YL; an apparent gap between the ends can be seen even in very early diplotene nuclei.

The appearance of several of the autosomal bivalents at early diplotene is highly characteristic. The largest bivalent forms a huge ring at this stage, the chiasmata being apparently completely terminalized; it contains no distinguishable heterochromatic segments. The second bivalent (that is, the one which usually forms a chiasma in the short arm) is largely heterochromatic, the degree of heteropycnosis being greater in one half than in the other, so that it forms a ring of four segments, two more strongly heteropycnotic than the other two. Since the chiasmata in this bivalent are terminalized we have not been able to decide with complete certainty whether it is the proximal or the distal half which is more strongly heteropycnotic. The remaining six bivalents usually each show a single distal chiasma. Several of them have proximal heterochromatic segments.

DISCUSSION

The neo-X : neo-Y mechanism of *P. beutenmulleri* does not differ in principle from a number of other similar cases, some of which have recently been discussed by King (1950). Such instances of X-autosome fusions are now known in a large number of unrelated genera of Acrididae belonging to several subfamilies (White, 1951, 1953). The main interest of these cases arises from the fact that we may expect them to form a graded series, the members of which show progressively increasing cytogenetic differences between XR and the neo-Y. Originally alike and homologous throughout their length, these elements have, in most cases, developed visible differences in length and in the distribution of recognizable heterochromatic and euchromatic segments.

In *P. beutenmulleri* XR and the neo-Y are still the same length, as far as we can judge. There is accordingly no evidence that any deletions, duplications or pericentric structural changes have occurred, or if such rearrangements have taken place they have only affected very short regions. On the other hand there are a number of small blocks of heterochromatin in the Y which are not present in XR. Thus, since the establishment of the fusion, the neo-Y has undergone a process of "heterochromatinization" which has not affected XR. Presumably small pairing segments still exist at the distal ends of these elements, in which a quasi-terminal chiasma is formed.

The sex trivalent of *P. morsei* resembles that of *P. brunneri*, as described by King and Beams, very closely. But there appear to be some differences. King and Beams figure YL ("A" in their figures) as longer than YR ("B"); whereas in *morsei* YR is longer than YL. Also, in *brunneri* YR seems to be more completely heterochromatic, while in *morsei* the distal part of this limb is largely euchromatic. Without actual material of *brunneri* for a direct comparison it is difficult to be certain of these differences, but a careful comparison of King and Beams' figures with our own preparations suggests that they are genuine. The difference in the relative lengths of YL and YR could have come about through a pericentric inversion, since the combined length of these arms (relative to the other chromosomes) seems to be about the same in the two species. Such an inversion would not be expected to lead to any difficulties at meiosis, provided that it left the small distal pairing segments intact. The heterochromatic blocks at the distal ends of XR and YL seem to be alike in *brunneri* and *morsei*.

The autosomal bivalents of *brunneri* and *morsei* are very similar, but the largest bivalent occasionally forms three chiasmata in *brunneri*. Also, King and Beams do not seem to have noted any chiasmata in the short arm of the second autosomal bivalent.

In general, it can be said that *beutenmulleri* has only acquired a single X-autosome fusion, whereas in the other two species an additional fusion between the originally acrocentric neo-Y and a second autosome has occurred, thereby giving rise to the multiple sex-chromosome mechanism. It is impossible to prove that the X-autosome fusion in *brunneri* and *morsei* is actually the same one that exists in *beutenmulleri*, but this seems probable on general grounds. *Brunneri* and *morsei* also possess a fusion between two autosomes, which has created a very large metacentric element. This autosomal fusion is not present in *beutenmulleri*. All three species show heterochromatinization of the neo-Y, while X_1R (XR in *beutenmulleri*) has remained largely euchromatic. In *morsei* the distal part of YR is likewise euchromatic, but in *brunneri* this region also seems to have undergone heterochromatinization.

SUMMARY

The grasshopper *Paratylotropidia beutenmulleri* possesses an X-autosome fusion which has converted the primitively XO sex chromosome mechanism of the male into a neo-X : neo-Y system. *P. morsei* and *P. brunneri* have an additional fusion which has given rise to an X_1X_2Y system. Some "heterochromatinization" of the chromosomal limbs included in the neo-Y's has taken place in all these species since the occurrence of the fusions which restricted these elements to the male line. It is believed that the heterochromatinization of the Y is somewhat more complete in *brunneri* than in *morsei*. The association of the sex chromosomes in this genus is believed to be confined to very short terminal pairing segments in which chiasmata

are formed and almost immediately terminalized (interstitial chiasmata have never been seen). This extreme distal localization and terminalization of the chiasmata is seen in a number of the autosomal bivalents as well, so that it may well have been characteristic of the autosomes which became included in the sex chromosome mechanism, prior to the occurrence of the fusion. In both *morsei* and *brunneri* a fusion between two autosomes has given rise to a huge metacentric element: in *morsei* this probably has recombination restricted to minute regions at the two ends; while in *brunneri* it apparently sometimes forms an interstitial chiasma.

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Vol. LXXXVII, No. 835 The American Naturalist July-August, 1953

NULLISOMIC ANALYSIS IN COMMON WHEAT*

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Common wheat, *Triticum aestivum* L. *emend* Fiori et Paoletti (= *T. vulgare*), has proved relatively unsatisfactory for genetic analysis by conventional methods. The number of good, useful genes discovered and linkages clearly established is small, although many genetic studies have been conducted with wheat. The chief difficulty is that wheat is a polyploid, specifically a hexaploid. Although it is an allo- rather than an autohexaploid, much of its genic material is duplicated or triplicated. Its three "genomes," or groups of seven chromosome pairs, come from related sources: the so-called A genome from einkorn, the diploid wheat; the B genome from an undiscovered, but certainly related, source; and the D (sometimes called C) genome from *Aegilops squarrosa*, a member of a closely related genus.

With the isolation of monosomic and nullisomic lines (Sears, 1939, 1944, and unpublished), the genetic analysis of wheat has been greatly facilitated. All the 21 nullisomics (with only 20 pairs of chromosomes instead of 21) that are possible in wheat have now been obtained in the variety Chinese Spring. Considerable progress has already been made in locating genes on the chromosomes using this new material.

Monosomic analysis has been used in *Nicotiana tabacum* with conspicuous success by Clausen (1941) and Clausen and Cameron (1944, 1950). Briefly the method consists of crossing a line carrying a particular gene with each of the 24 different tobacco monosomics. Part of each F_1 population is monosomic, and if the gene is recessive, the monosomic plants of the critical F_1 family are all of the recessive phenotype. If the gene is dominant, F_2 populations are grown from monosomic F_1 plants, and the critical F_2 family shows no recessive segregates.

BREEDING BEHAVIOR OF WHEAT MONOSOMICS

Nullisomic wheat plants produce only nullisomic offspring when selfed. Monosomics, however, yield three types of offspring, disomic, monosomic, and nullisomic, in frequencies which vary somewhat with the chromosome concerned. As indicated in table 1, about 75 per cent of the female gametes have only 20 chromosomes. This deviation from the theoretical 50 per cent

*Journal Paper No. 1353 of the Missouri Agricultural Experiment Station. Cooperative work between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Field Crops Department, University of Missouri. Supported in part by funds obtained under Bankhead-Jones Project SRF 2-95, "Combining in Wheat the Disease Resistance and Other Desirable Characters of Related Grass Species."

is due to the irregular behavior and resultant frequent loss of the unpaired monosome at the reduction division. On the male side the *functioning* gametes predominantly carry 21 rather than 20 chromosomes, because pollen competition strongly favors the 21-chromosome pollen. The 21-chromosome male gametes account for from about 90 per cent to more than 99 per cent of the fertilizations. The frequency of nullisomics obtained, which depends very largely upon the frequency of functioning 20-chromosome pollen, varies from about 10 per cent for nullisomic III (Sears, 1944) to about one per cent for several other nullisomics.

TABLE 1

FREQUENCIES OF FUNCTIONING 21- AND 20-CHROMOSOME GAMETES PRODUCED BY PLANTS OF A TYPICAL MONOSOMIC OF WHEAT, AND THE RESULTING FREQUENCIES OF DISOMIC, MONOSOMIC, AND NULLISOMIC OFFSPRING

	21-chromosome pollen 96 per cent	20-chromosome pollen 4 per cent
21-chromosome eggs 25 per cent	21" plants 24 per cent	20" 1' plants 1 per cent
20-chromosome eggs 75 per cent	20" 1' plants 72 per cent	20" plants 3 per cent

Totals: 21" plants 24 per cent
20" 1' plants 73 per cent
20" plants 3 per cent

METHODS OF ANALYSIS IN WHEAT

Four general methods have been used in wheat for determining on which chromosomes genes are located: (1) ascertaining that the effect of a particular gene is absent in a particular nullisomic; (2) growing F_2 populations from crosses with each of the 21 nullisomics and determining which F_2 family segregates abnormally; (3) analyzing selected F_2 plants in the F_3 generation; and (4) substituting the individual chromosomes of other varieties into a standard variety.

1. *Locating Genes by Absence of Expression in Nullisomic:* Certain dominant genes in the variety Chinese Spring, in which the complete nullisomic series is available, can be located simply by observing the absence of their effect in the proper nullisomic. Thus, Chinese has a dominant gene for red seeds, and this has been located on chromosome XVI by the fact that when nullisomic XVI produces seeds, they are white. Similarly, chromosomes VIII and X each carry a dominant inhibitor of awns, as shown by the increased awn development in nullisomics VIII and X.

There is a class of dominants in wheat which cannot be located in this way, because the critical nullisomic still shows the dominant phenotype. An example is the dominant non-*sphaerococcum* gene, carried by chromo-

some XVI of the variety Chinese (Sears, 1947). The recessive allele of this gene determines the distinctive characteristics of *T. sphaerococcum* Perc. No significant tendency to resemble *T. sphaerococcum* can be observed in Chinese nullisomic XVI. Similarly, chromosome III of Chinese carries the dominant allele of the recessive virescent gene described by Neatby (1933), yet nullisomic III shows no deficiency of chlorophyll.

The corresponding type of recessive gene, when present in Chinese, can be located by simple observation of nullisomics or even monosomics. These recessives are ineffective in the hemizygous condition (that is, in one dose, with no dominant allele present). For example, mono-IX (and nulli-IX) of Chinese are non-squarehead and speltoid due to inability of the recessive squarehead and speltoid-suppressing genes on chromosome IX to express themselves in single dose.

It would be feasible to locate these hemizygous-ineffective recessives as well as normal dominant genes in a variety other than Chinese by first obtaining nullisomics there. This would involve crossing to the Chinese nullisomics and backcrossing to the other variety for several generations, using only monosomic offspring in making each backcross. Usually some other method of gene location would be preferable.

2. *Locating Genes by Studies of F_2 Populations:* Ordinary dominant genes in varieties other than Chinese are located by crossing to each of the 21 nullisomics and observing the ratios obtained in the F_2 populations. With a simple dominant gene, the critical family, instead of segregating the customary 25 per cent recessives, usually yields only 1 to 5 per cent depending on the particular chromosome concerned, with 10 per cent being about the maximum. The deviation from the usual 3:1 ratio is due to the fact that both disomics and monosomics carry the dominant allele, in double and single dose, respectively, and only the nullisomics are free to express the non-dominant or recessive characteristic. As has previously been noted (table 1 and accompanying text), nullisomics normally appear in frequencies of only 1 to 10 per cent among the offspring of monosomics.

The location of two simple dominant genes using the F_2 method was reported by Unrau (1950). In the F_2 from a cross with nulli-I, a ratio of 528 red-chaffed to 38 white-chaffed plants was obtained instead of the expected 3:1; and from the cross with nulli-XX, 220 compact-spiked to 11 lax-spiked were observed.

It is not necessary in using the F_2 method to rely entirely on obtaining a ratio deviating significantly from 3:1, for a perfect coincidence of nullisomics and recessives is expected in the critical family. By checking for this coincidence, it is possible to obtain a significant result with F_2 populations numbering as few as 20 plants each. For example, F_2 families of 20 to 24 individuals each were grown from crosses to the nullisomics of the pubescent-glumed variety Indian. In 20 of the 21 F_2 populations, there was no significant deviation from the ratio 3 pubescent: 1 non-pubescent, and the non-pubescent plants in each family included some non-nullisomics. In the F_2 involving chromosome XIV, however, the segregation was 19 non-

nullisomic pubescent: 1 nullisomic non-pubescent. These data are conclusive in placing the pubescent gene on chromosome XIV.

The hemizygous-ineffective recessives previously referred to can be located by the study of F_2 populations, but not simply by observation of F_2 ratios. These genes in the critical F_2 families from crosses with the nullisomics express themselves only in the disomic segregates. Both the monosomics and the nullisomics show the dominant phenotype. Since the disomics constitute approximately one-fourth of the population (see table 1), the critical F_2 does not differ significantly from the other F_2 families in the proportion of recessive segregates. Cytological study of the recessives, however, reveals the critical family by establishing that in this family all the recessives are disomic. In the other families only about one-fourth of the recessives are disomic.

The previously mentioned *sphaerococcum* gene provides an example of the locating of hemizygous-ineffective recessives. Ellerton (1939) showed this gene to be a simple recessive, and nullisomic analysis has proved it to be located on chromosome XVI (Sears, 1947). In the F_2 family involving chromosome XVI, there were 6 *sphaerococcum*-like segregates and 14 non-*sphaerococcum*, including one nullisomic. When cytological studies were made of this family, it was found that all of the 5 *sphaerococcum*-like plants examined were disomic. This could have happened by chance only about once in 1000 times.

More complex genetic situations, involving two or more duplicate or complementary genes located on different chromosomes, are also subject to nullisomic or monosomic analysis, using the F_2 method. Clausen and Cameron (1944, 1950) described the location of duplicate genes in *Nicotiana*. There were two critical F_2 families, which segregated in 3:1 ratios rather than 15:1. Similarly, Unrau (1950) found in wheat that one of two duplicate genes for winter growth habit was located on chromosome IX. For two complementary dominant genes, it is necessary to distinguish an approximate 3:1 ratio from 9:7 (a test requiring at least 95 offspring), and with three or more duplicate or complementary genes, distinctions requiring still larger numbers of plants would be involved. Larson (1952), relying mainly on F_2 data, located four genes affecting solidness of stem in the variety S-615.

Where two or more genes with duplicate or complementary effect are located on a single chromosome, the F_2 method will show at once which chromosome carries them. This is well illustrated by the results with resistance to stem-rust race 56 in the variety Timstein (Sears and Rodenhiser, 1948). It was found that an approximate 9:7 ratio of resistant to susceptible plants was obtained in all the F_2 populations grown except the one involving chromosome X. Here only two susceptible plants appeared among 124 tested, pointing to chromosome X as being the bearer of both the complementary dominant genes. Linkage between the two genes was indicated by a near-significant departure from 9:7 in the normally segregating families.

3. *Locating Genes by Studying Selected F_3 Populations:* If it is not possible to score the F_2 population, or if it is desired to locate several genes

requiring test procedures, it may be advisable to study F_3 progenies from three or four disomic individuals selected by cytological observation in the F_2 generation. These F_2 plants are handled so as to insure maximum seed setting. Where the critical chromosome is involved, all of the disomics will be found to have been homozygous for the gene under study. The odds against obtaining three homozygotes by chance are 63:1, and against obtaining four, 255:1.

4. *Locating Genes by Making Chromosome Substitutions:* If accurate classification of segregants is difficult or impossible, as when certain types of disease resistance are involved, or if minor or modifying factors are operating, it may be desirable, or even necessary, to use the method of single-chromosome substitutions for locating genes. In this method each chromosome of the variety chosen is transferred intact to the control variety by means of backcrosses to the respective nullisomics, and its effect is then measured with relative precision.

The process involves first making crosses of the nullisomics with the variety to be analyzed. The monosomic F_1 plants are next backcrossed as males to the respective nullisomics. From 90 to 99 per cent of the offspring are monosomic, and one of these monosomics is again backcrossed to the nullisomic. By the fifth backcross generation, about 97 per cent of the other 20 chromosomes of the non-recurrent variety have been eliminated, but the one chromosome being controlled is still present intact, having had no opportunity to pair and cross over with a homologous chromosome. If 97 per cent is considered sufficient purity, a B_5 monosomic plant is now allowed to self-pollinate, and about one-fourth of the resulting offspring are disomic for the substituted chromosome. The disomics now constitute essentially a new line with one particular pair of chromosomes substituted for the corresponding pair of the standard variety.

It is now possible to determine rather accurately what genes the substituted chromosome carries. The line is uniform, with little or no more variability than the standard variety. It is normal with respect to chromosome constitution, and in nearly every instance is fully fertile. Seed can be obtained in unlimited quantities for test of the chromosome for its content of genes for resistance to any disease or insect desired. Hemizygous-ineffective recessive genes exert their effect in this material. To ascertain whether a single gene may be responsible for a chromosome's action, crosses may be made with the standard variety, and only genes on the one chromosome will segregate.

As an example of the use of this method, chromosome X from the variety Timstein has been transferred to the variety Chinese, and it has now been possible with a minimum of inoculation work to establish that this chromosome controls resistance to every one of the several stem-rust races tested.

THE USE OF ISOCHROMOSOMES AND TELOCENTRICS

Due to difficulties with reduced vigor and fertility, it is usually not practicable to use the nullisomics in making crosses. In actual practice, only

nulli-I, nulli-VII, and nulli-XXI are maintained as nullisomic lines, and only nulli-VII and nulli-XXI are consistently used in crosses. For the remaining 19 chromosomes, monosomics are used instead of nullisomics. On the female side they produce about 75 per cent of the desired 20-chromosome gametes. It is usually necessary to check the constitution of the F_1 plants cytologically when monosomics are used.

In the production of substituted lines (method 4 of genetic analysis) ordinary monosomics can not be used because of the possibility, apparent in table 1, of obtaining monosomic offspring deriving their monosome from the female parent instead of the male. Abnormal monosomes are available, however, which are recognizable cytologically. They are telocentric chromosomes, which have lost one entire arm, and isochromosomes, of which one arm has been lost and the other arm duplicated (Sears, 1946). Plants monosomic for these modified chromosomes can safely be used instead of nullisomics in the production of substituted lines.

EFFECT OF TRANSLOCATIONS

Reciprocal translocations are not uncommon in varieties of hexaploid wheat. Taking Chinese Spring as the standard, the variety Thatcher, for example, has a translocation which involves chromosomes IV and X. Poso (a variety of *T. compactum*) has a translocation involving chromosomes V and VII, and Indian has one involving III and VII.

The effect of translocations on the transfer of individual chromosomes from other varieties into Chinese (method 4 of genetic analysis) may be illustrated by results with Thatcher. When Thatcher is crossed to nulli-IV Chinese, the F_1 plants have 19 meiotic bivalents and a chain of three chromosomes. This chain is composed of chromosome X from Chinese and two Thatcher chromosomes which may be represented as IV-X and X-IV. Disjunction from the chain is almost always such that Chinese-X goes to one pole and Thatcher IV-X and X-IV go to the other. In the backcross to nulli-IV the gametes carrying IV-X and X-IV are selected, and the B_1 plants again have the chain of three. Through crossing over in the F_1 and backcross generations, the portions of IV-X and X-IV homologous to Chinese X are gradually replaced by Chinese chromatin. When backcrossing is completed and a selfed population grown, the plants selected with 21 pairs have the Thatcher chromosome arrangement (that is, are translocation homozygotes relative to the Chinese standard), but the substituted Thatcher chromosome material is the equivalent of a pair of Chinese chromosome IV's divided between two different pairs of chromosomes.

In the transfer of the nullisomic condition from Chinese to other varieties (method 1 of genetic analysis), the complications due to translocations are also relatively minor. Using nulli-IV and Thatcher again as an example, the same chain of three is present throughout the series of backcrosses, which in this case are made to Thatcher. The member of the chain originally from Chinese is retained from generation to generation, but the genes it carries are gradually replaced by crossing over. The nullisomics finally

produced by selfing a B_n plant have the Chinese chromosome arrangement, with no ring or chain.

Anything which interferes with crossing over in the chain of three will of course make gene replacement more difficult and perhaps necessitate the growing of additional backcross generations. Interference of some sort may occur in the chains, but this will only involve regions near the point of interchange. Even if gene replacement is incomplete, there is little danger of errors in locating genes, for the line obtained following crossing, in the case of Thatcher, to Chinese nullisomic X can be utilized as a check.

DISCUSSION

One type of gene met with in these studies is so unusual as to deserve further mention. This is the recessive gene that is ineffective when hemizygous, and whose dominant allele shows no dosage effect. Although proper dosage data are available for relatively few genes in organisms like maize and Drosophila, from which most of our knowledge of genetics has come, it seems safe to say that extremely few of their genes are of this type. In wheat, on the other hand, four hemizygous-ineffective recessives have been identified among only about twenty genes thus far studied in detail.

It appears likely that the reason for the existence of this unusual type of gene in wheat is the polyploid nature of the wheat plant. In a diploid organism a gene that has a positive effect is almost always dominant, because one dose of the gene is adequate to insure full expression. In a hexaploid like wheat, however, the gene is subject to the action of modifiers not only from its own genome, but from two additional related genomes as well. It would not be surprising, then, if some genes in wheat were unable to reach their threshold of expression in a single dose. Such genes would be recessive, and they would be ineffective when hemizygous.

Although no definite dosage effects have yet been observed for the dominant alleles of the four hemizygous-ineffective recessives studied, the data are not extensive enough to prove that dosage is completely without effect on these alleles. Indeed, only for the non-*sphaerococcum* and the non-virescent dominants has the full dosage series extending from nullisomic to tetrasomic been available. In the case of *sphaerococcum*, four doses of the chromosome carrying the dominant gene have little, if any, effect on any of the *sphaerococcum* characters except spike density. The density effect need not be due to the gene in question, since genes affecting density are numerous and widely scattered among the chromosomes. The dominant allele of virescent shows a slight reduction in chlorophyll from zero to one dose, but this may be a secondary consequence of the great increase in plant size from nullisomic to monosomic.

SUMMARY

Four methods are outlined for using nullisomics and monosomics in wheat to locate genes on chromosomes:

1. Absence of effect of a particular gene in a particular nullisomic. This applies to ordinary dominants and certain recessives in any variety in which nullisomics are available.

2. Abnormal segregation in F_2. A dominant gene in another variety gives an aberrant F_2 ratio following crossing with a line nullisomic for the chromosome on which the gene is located. A hemizygous-ineffective recessive appears in normal frequency in F_2, but is confined to disomic individuals.

3. Homozygosity of F_2 disomics. Study of F_3 populations shows in which F_2 family all the disomic plants were homozygous for the gene in question.

4. Gene content of substituted lines. Each chromosome of the variety under test is transferred separately to a standard line, where its effects indicate what gene it carries.

Certain recessive genes in wheat are ineffective in single dose (hemizygous), and their dominant alleles show no dosage effect. They are positively acting genes that require two doses to reach their threshold of expression.

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Vol. LXXXVII, No. 835 The American Naturalist July-August, 1953

A CORRELATION OF OBSERVATIONS SUGGESTING A FAMILIAL MODE OF MOLECULAR EVOLUTION AS A CONCOMITANT OF BIOLOGICAL EVOLUTION*

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A number of lines of evidence which have developed in recent years point toward concepts suggesting more explicitly a mode of evolution at the molecular level. One essential proposition to be considered on the basis of the available evidence is that a limited randomness of biosynthesis in the individual organism tends to confer a selective advantage upon that organism in its competition for survival.

This hypothesis originated indirectly from the extensively unsuccessful attempts of physical chemists to establish preparations of protein as homogeneous. The history in this field includes numerous instances in which protein preparations were adjudged pure by one or more criteria; later investigations cast doubt on such conclusions (cf., e.g., Anderson and Alberty, 1948). Some of the workers in this field have therefore been led to suspect that proteins are not produced in an organism as invariable entities, but that variations in structure may be synthesized often enough that the probability of obtaining a homogeneous protein is either low or nonexistent (Williams, 1951).

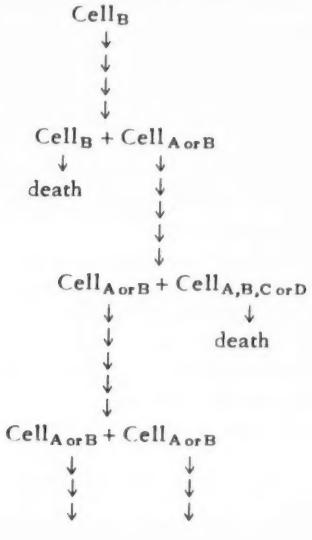
Another type of evidence may also be interpreted as indicating that molecules from the same organism conform to a sibling type of relationship. Such evidence has been obtained from the molecular diversities found in, for example, penicillin (Clarke, 1949); gramicidin (Gregory and Craig, 1938); bacitracin (Craig, Gregory, and Barry, 1949); vitamin E (Karrer and Fritzsche, 1938); insulin (Harfenist and Craig, 1952); cf. also data on γ-globulin molecules (Cann, Brown, Singer, Shumaker, and Kirkwood, 1951; Porter, 1950; Stormont, Owen, and Irwin, 1951). Each of these examples illustrates or adds to the evaluation of a limited diversity in biosynthesis. The last, particularly, permits an understanding of the wide utilities of mammalian antibodies (Landsteiner, 1946).

The selective advantages conferred upon an organism by its ability to synthesize sibling molecules should stem in part from the fact that some of the variant molecules may be more effective than their parent types in a given situation. The possession of a number of sibling molecules may however also be expected to provide increased versatility of response to an ever-changing environment in which the critical demands do not remain constant. Mutation away from rigid and invariant replicability of molecular

*Journal Paper No. J-2245 of the Iowa Agricultural Experiment Station, Project No. 1111, supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service and the Rockefeller Foundation.

synthesis should in at least some cases thus confer selective advantage. On first consideration it might appear that such a process would lead to a biologically uneconomically large number of syntheses; this problem poses its own answer, however. When the number of syntheses becomes uneconomically large, the organism has attained a stage at which it now suffers a considerable measure of selective disadvantage. The two pressures, one to expand synthesis, one to limit it, should lead to a relatively balanced state in which syntheses possess a limited randomness but are neither immutable nor promiscuous. Such shifts in range of synthesis would of course need, in conformity with conventional genetic thought, to be gene-controlled.

Such an overall mechanism may be considered in more detail for its effect within many generations, by a hypothetical example. In this example, attention is focussed upon the production by a microbial cell of a trypsin molecule to aid in its nutrition. This particular trypsin may be designated trypsin B and it would represent one of four hypothetical trypsins (A, B, C, or D). Variation in efficiency of the trypsin is also indicated by the subscripts, each being most efficient for a particular type of proteinaceous nutrient. $Cell_B$ producing $trypsin_B$ divides to give $cell_B$ and $cell_{A\,or\,B}$ in one of the generations. This generation involves some randomized biosynthesis stemming from the synthesis of $trypsin_B$. The cell possessing A and B abilities is more versatile. If the nutrient in the environment becomes limiting this cell survives because of its greater versatility. $Cell_B$, unable to compete successfully in this situation, perishes. In a later generation, the A, B, C, or D type which arises cannot compete with the A or B type since it is uneconomically random in its synthesis so that it is also at a disadvantage in competition with $Cell_{A\,or\,B}$ (this assumes also that in the environment in this generation C or D is not superior to A and B). The



rates of mutation would influence at least the attainment of such an equilibrium and they should be many orders of magnitude lower than in the hypothetical flow-sheet presented.

Randomized biosynthesis within an individual organism, and its consequences as set out above, could explain current knowledge which indicates that more distantly related biological forms contain more distantly related molecular structures. Selection of the type illustrated in the flow-sheet could lead from one synthesis (as $trypsin_B$) to another (as $trypsin_A$). The greater the span of molecular evolution, the greater should be the opportunity for more far-flung biological evolution; the reverse relationship should also hold. Diversity of synthesis within an organism should thus be part of the mechanism for the development of diverse syntheses between organisms, and through these links evolution can progress because heritable replicative chemical synthesis is not immutable within the organism. In a survey of the fungal antibiotics, Brian, 1951, has found that closely related species tend to produce the same antibiotics or groups of antibiotics, whereas more distantly related fungi tend to produce different antibiotics. An evolutionary relationship is thus emphasized in one way by the antibiotics, which may be looked upon as weapons in the competitive struggle for existence among different micro-organisms. Those antibiotics which are most effective would contribute to the survivability of the producing organism; the thriving organism would in turn tend to perpetuate the antibiotics which its parent cells produced.

The greater spread of biosynthetic spectrum with a larger segment of biological spectrum is observed not only for the antibiotics but in other instances as, e.g., the cases of tobacco mosaic virus (Stanley, 1947), hemoglobins (Porter and Sanger, 1948), and the more distant relationship of hemin and chlorophyll as contrasted to the close relationship of chlorophyll a and chlorophyll b (Karrer, 1950). These examples illustrate or add to an evaluation of the fine chemical differences which can conceivably support the fingerprint diversity of inheritance. Evolution at the molecular and biological levels should thus protect and support each other through a mechanism which stems from the limited flexibility of synthesis.

Viewed against all of the considerations presented, the heterogeneity which is the dismay of the protein chemist attempting to solve purification problems may be the very basis for his existence as a human being and his singularity as an individual.

The evidence for the correlations presented is of course suggestive rather than conclusive, and rests at present essentially on observation, as originally did the phylogenetic theory of evolution. Since the generalizations suggest a number of consequences, they may be worthy of critical appraisal and attempts to determine if they also possess survival value. In this respect, earlier drafts of this paper have received constructive criticism from a number of investigators. In particular the aid of Dr. Daniel E. Atkinson is gratefully acknowledged.

SUMMARY

Evidence pointing toward the synthesis of families of molecules within the organism is discussed. This view has suggested the hypothesis that a limited randomness of molecular biosynthesis in the individual organism confers a selective advantage upon the organism. This advantage is due to the production of some superior molecules, and to the versatility which is provided. The probability that a limited diversity of synthesis within the individual organism leads to related molecules in organisms of different types is also suggested.

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Vol. LXXXVII, No. 835 The American Naturalist July-August, 1953

IS RH FACING A CROSSROAD? A CRITIQUE OF THE COMPENSATION EFFECT

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Shortly after the Rh agglutinogen was discovered, Haldane (1942) recognized that it is a case of selection against heterozygotes through the death from erythroblastosis fetalis of babies born to recessive mothers. He pointed out that the present American population with its high recessive rh frequency is probably in a very unstable situation and that the rh gene is in the process of being eliminated. A little later, R. A. Fisher (Race, 1944) suggested that the frequency of rh in the population may be maintained at the present level by the tendency of mothers to replace lost children by continuing to have more babies to bring their family to the normal size and even larger. These surviving children are rh-negative and they compensate or even overcompensate the loss of rh gene through the death of their heterozygous sibs. The simple hypothetical cases considered by Spencer (1947) in an earlier issue of this journal showed that the compensation effect would actually increase the rh frequency in the population if it is higher than a certain minimum value at that time. His particular numerical examples will be generalized in the subsequent analysis. Recently, Glass (1950) showed with a unique set of data from the Baltimore Rh Typing Laboratory that the compensation tendency on the part of rh-negative mothers is a reality, not just a hypothesis. Thus, he found that the mean number of *living* children per rh-negative mother is 1.454, that per sensitized rh-negative mother is 1.62 and that per Rh-positive mother is only 1.378 for the American Whites. It is the reality of the compensation phenomenon that instigates the following discussion.

Since we shall only deal with the principal positive and negative alleles as did the authors cited above, we shall use A for Rh (dominant), a for rh (recessive) and p & q to denote their respective frequencies in a panmictic population where $p + q = 1$. The selection against heterozygous children born to recessive mothers and the tendency on the part of such mothers to have additional births (recessive children) to replace their lost ones are put into algebraic terms in table 1 in which s is the selection coefficient and t is the compensation coefficient (both positive fractions). Now, let W denote the "fitness" or the surviving value of a genotype and $\bar{W}$ be the average fitness of the population as a whole. From the bottom row of table 1 (total offspring) we see that

$$W_{AA} = 1, \quad W_{Aa} = 1 - \tfrac{1}{2}sq, \quad W_{aa} = 1 + tpq \qquad (1)$$

and

$$\bar{W} = 1 - spq^2 + tpq^3.$$

TABLE 1

THE SELECTION AGAINST HETEROZYGOTES BORN TO RECESSIVE MOTHERS WHO COMPENSATE THEIR LOSS BY HAVING MORE RECESSIVE OFFSPRING

Mother × Father	Frequency	Offspring			Relative size of family
		AA	Aa	aa	
AA × all	p^2	p^3	p^2q	0	1
Aa × all	$2pq$	p^2q	pq	pq^2	1
aa × AA	p^2q^2	0	$p^2q^2(1-s)$	0	$1-s$
aa × Aa	$2pq^3$	0	$pq^3(1-s)$	$pq^3(1+t)$	$\frac{(1-s)+(1+t)}{2}$
aa × aa	q^4	0	0	q^4	1
Total	1.00	p^2	$2pq - spq^2$	$q^2 + tpq^3$	

On account of the selection and the simultaneous compensation, the frequency of *a* will change from q to

$$q' = \frac{1}{\bar{W}}\{q - \tfrac{1}{2}spq^2 + tpq^3\} \tag{2}$$

in the next generation so that the "increment" per generation is

$$\Delta q = q' - q = \frac{pq^2}{\bar{W}}\{-\tfrac{1}{2}s + (t+s)q - tq^2\}. \tag{3}$$

Obviously, $\hat{q} = 0$ and $\hat{q} = 1$ are two stable equilibrium points. But the point given by the root of the quadratic equation $tq^2 - (t+s)q + \tfrac{1}{2}s = 0$ is an *unstable* one, its value being

$$\tilde{q} = \frac{t + s - \sqrt{t^2 + s^2}}{2t} \tag{4}$$

where the tilde indicates its instability. The other root is greater than unity and thus of no interest to us.

The instability of an equilibrium point does not always receive adequate attention from some geneticists. It should be clearly borne in mind that an unstable equilibrium value acts like a "repulsive" point to gene frequency which moves away from it, not toward it. Therefore, an unstable equilibrium value can never be "reached" under the given scheme of selection.

The values of $\tilde{q}$ for various magnitudes of s and t have been given in table 2 for the convenience of discussion. It should be noted that $\tilde{q}$ is determined by the *relative* rather than absolute magnitudes of s and t. Thus, when $s = t$, (4) reduces to $(2 - \sqrt{2})/2 = .2929$; when $s:t = 4:3$, $\tilde{q} = \tfrac{1}{3}$, and so on. The actual amount of increment Δq of course depends upon their absolute values. The examples considered by Spencer (1947) are particular cases of these two relative magnitudes of s and t. Thus, his first example

that all heterozygotes born to recessive mothers die but are fully replaced by recessive children (when the father is heterozygous) is the case $s = t = 1$. If the existing q is .30, only slightly higher than .2929, it would be increased to .30066 in the next generation according to (2). On the other hand, if $q = .29$ at that time, it would be decreased to .28974 in the next generation. It follows that the compensation mechanism cannot maintain the frequency of the recessive gene at a certain *constant* high level for a large number of generations; it may hasten q to reach the point 1 or slow down its rate of reaching 0, depending on whether the existing q happens to be above or below the "repulsive" point.

It is also worth while to note that

$$\text{when} \qquad t >> s, \quad \tilde{q} \doteq \frac{s}{2t}\left(1 - \frac{s}{2t}\right) \doteq \frac{s}{2t} \tag{4T}$$

$$\text{when} \qquad s >> t, \quad \tilde{q} \doteq \frac{2s - t}{4s} \tag{4S}$$

where the symbol >> reads "much larger than." Note that when $t = 0$, $\tilde{q} = \frac{1}{2}$, and our formula (3) reduces to an expression given by Haldane (1942).

In brief, the relative magnitudes of s and t determine the unstable equilibrium point. The effect of compensation is to lower the value of that critical point so that an existing q may have a chance to increase even if its value is below $\frac{1}{2}$ (the required minimum without compensation).

Now, let us consider the present American White population with existing $q = .39$ approximately and s between .025 to .100 as estimated by Haldane

TABLE 2

VALUES OF $\tilde{q} = \dfrac{t + s - \sqrt{t^2 + s^2}}{2t}$

Selection coeff. s	t (compensation coefficient) .01	.02	.03	.04	.05	.06	.07	.08	.09	.10
.01	.2929	.1910	.1396	.1096	.0901	.0764	.0663	.0586	.0525	.0475
.02	.3820	.2929	.2324	.1910	.1615	.1396	.1228	.1096	.0989	.0901
.03	.4189	.3486	.2929	.2500	.2169	.1910	.1703	.1535	.1396	.1280
.04	.4384	.3820	.3333	.2929	.2597	.2324	.2098	.1910	.1751	.1615
.05	.4505	.4037	.3615	.3246	.2929	.2658	.2427	.2229	.2058	.1910
.06	.4586	.4189	.3820	.3486	.3190	.2929	.2700	.2500	.2324	.2169
.07	.4644	.4325	.3974	.3672	.3398	.3150	.2929	.2731	.2554	.2396
.08	.4688	.4384	.4093	.3820	.3566	.3333	.3121	.2929	.2754	.2597
.09	.4723	.4451	.4189	.3939	.3704	.3486	.3284	.3099	.2929	.2773
.10	.4750	.4505	.4267	.4037	.3820	.3615	.3424	.3246	.3081	.2929

(1942). The value of t may be roughly estimated from the data given by Glass (1950). Thus, for sensitized rh-negative mothers, we have 1.62/1.378 = 1.176. If this ratio is unity, it means s = t. If we take s = .05 in the equation $(2 - s + t)/2 = 1.176$, we obtain t = .40 approximately. For rh-negative mothers in general, the observed ratio is 1.454/1.378 = 1.055 while its theoretical size is (see table 1)

$$\frac{q^2 - s(p^2q^2 + pq^3) + tpq^3}{q^2} = 1 - sp + tpq.$$

Using q = .39 and s = .05 as before and equating it to 1.055, we obtain t = .36 in good agreement with the preceding estimate. If t is larger than s at all, the present value of q, being higher than .2929 (table 2), will continue to increase. One important point brought out by Glass (1950) is that when the average size of sibship is large in the general population, the compensation effect would be much lessened because the recessive mothers then could not have many more children than the average family. Now, before the advent of modern medical science and modern "civilization," the value of s must have been comparatively large while that of t small owing to the then larger size of families. Table 2 and (4S) show that the unstable equilibrium point should then be near to ½. Hence, it seems possible that q had been decreasing all along until the last few decades when the relative magnitudes of s and t have been reversed and the repulsive point drastically lowered (i.e. moved from the lower left to the upper right of table 2). Consequently, the movement of q changed its direction too.

The writer limits himself to the discussion of the compensation effect alone in this paper. The origin of the high frequency of rh is another question and various hypotheses have been advanced (Haldane, 1942; Spencer, 1947) to that effect. As far as the future course of rh is concerned, it seems that nothing could be said definitely—it all depends upon the future status of s and t as well as on which side of $\tilde{q}$ the future rh will find itself. Should medical science be able to reduce s to zero in the future and compensation automatically disappears, rh may become stabilized where it happens to be. Apparently, either we or the rh will be in a state of confusion or indecision for some time to come.

SUMMARY

The compensation on the part of rh-negative mothers who tend to replace their lost (heterozygous) children by having more (recessive) births cannot lead to a stable equilibrium value of rh frequency.

The compensation coefficient (t) has been estimated to be roughly 0.36 to 0.40 for American Whites, being much larger than selection intensity (.025 to .10). Although it seems to be on the increase at present, the future direction of change in rh frequency and its ultimate fate in the population is still indefinite, depending upon the future status of selection and compensation.

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Vol. LXXXVII, No. 835 The American Naturalist July-August, 1953

THE PROBLEM OF ESTIMATING THE NUMBER OF LOCI DETERMINING QUANTITATIVE VARIATION IN HAPLOID ORGANISMS*

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The problem of the estimation of the number of effective factors responsible for quantitative variations in diploid organisms has been considered by several authors. The fundamental equations for such an estimation from parental, F_1, F_2, and backcross means and variances were derived by Wright (1934), and corrections for linkage have been devised by Dempster and Snyder (1950) and by E. L. Green (personal communication). The whole problem has been discussed in detail by Mather (1949).

The problem of estimation in a haploid organism arose in this laboratory in connection with an investigation of the inheritance of tyrosinase in strain 15300 (albino-2), *Neurospora crassa*. The forms derived in this note are of general application, but are discussed in specific connection with Neurospora. Following Dodge (1931), the upper case F will symbolize zygotic generations, and the lower case f will be applied to haploid generations.

In this treatment the usual assumptions are made:

1. There are at each locus two alleles, one making a positive contribution to the character in question and the other a negative contribution.
2. Parent A possesses all the plus alleles.
3. Parent B possesses all the negative alleles.
4. Each locus assorts independently.
5. The contribution (plus or minus) of each factor to the magnitude of the character is equal and additive.

In the case of diploids a lack of dominance must be assumed; in haploids this assumption is unnecessary.

In haploid organisms where all four products of meiosis are recoverable, the equations derived for use with diploid organisms may be applied by estimating values for the transient zygotes from their haploid products. In general, the value of a zygote will be equal to half the sum of the values exhibited by its four meiotic products. In organisms like yeast, where diploids as well as haploids may be propagated vegetatively, the assumptions of such a procedure might be tested directly.

In applying the diploid equations to Neurospora, the following procedure would be appropriate. The difference between the mean measurements of the two parental strains would be doubled and used as an estimate of the

*Supported by a research grant from the National Institutes of Health, U. S. Public Health Service.

parental zygotic difference. After mating the parental strains, the value of each F_1 zygote would be estimated by halving the sum of the measurements of the four different ascospore cultures from a single ascus. The variance of these F_1 zygotic values would be taken as an estimate of environmental variance. From each ascus a random selection of one f_1 ascospore would be made. Matings among these would yield f_2 asci, from which F_2 zygotic values could be determined as above. Matings with the parental cultures would yield backcross asci from which backcross zygotic values could be obtained in the same fashion. In the case of F_2 and backcross zygotes, only one ascus per mating would be analyzed. From the parental difference (zygotic) and the F_1, F_2, and backcross variances, estimates of the number of loci would be calculated. Transformation of scale to correct for non-additivity and correction for linkage could be made if desirable.

It is far more convenient, however, to estimate the number of loci directly from the haploid material. If the above assumptions are made, suitable equations are easily derived. Let

μ_A = mean of parent A measurements
μ_B = mean of parent B measurements
$|d|$ = contribution (+ or −) of a single allele to the magnitude of the character as defined by assumption five.
k = total number of loci
$\Delta = \mu_A - \mu_B = 2kd$

Consider the contribution of a single pair of alleles to the f_1 generation:

Constitution of f_1:	½ A	½ a
Contribution to character:	½ (+d)	½ (−d)

The contribution of such a pair of alleles to the f_1 mean would equal zero. Their contribution to the heritable variance of the f_1 would therefore equal

$$_{H_1}\sigma_{f_1}^2 = \tfrac{1}{2}d^2 + \tfrac{1}{2}d^2 = d^2.$$

For k independently assorting loci, the mean

$$M_{f_1} = 0,$$

and the heritable variance

$$_{H}\sigma_{f_1}^2 = kd^2.$$

Since

$$d^2 = \frac{\Delta^2}{4k^2},$$

then

$$_{H}\sigma_{f_1}^2 = \frac{\Delta^2}{4k}$$

and

$$k = \frac{\Delta^2}{4_H\sigma_{f_1}^2}.$$

It will be noted that $_H\sigma_{f_1}^2$ is twice the variance of zygotic F_2 generations

$$\left({}_H\sigma_{F_2}^2 = \frac{kd^2}{2} \quad \text{and} \quad k = \frac{\Delta^2}{8_H\sigma_{F_2}^2} \right).$$

In a similar fashion, formulae for the estimation of k from backcross generations may be derived. Consider a single locus:

Constitution of f_1: $\frac{1}{2}$ A $\frac{1}{2}$a.

If f_1 is backcrossed to parent A to produce the backcross generation (haploid) b_A:

Mating	*Offspring*	
$\frac{1}{2}$ A × A	$\frac{1}{2}$ A	0 a
$\frac{1}{2}$ a × A	$\frac{1}{4}$ A	$\frac{1}{4}$ a
Constitution of b_A:	$\frac{3}{4}$ A	$\frac{1}{4}$ a
Contribution to character:	$\frac{3}{4}$ (+d)	$\frac{1}{4}$ (−d)

The contribution of such a pair of alleles to the b_A mean would equal

$$_1M_{b_A} = \tfrac{1}{2}(\tfrac{3}{4}\,d - \tfrac{1}{4}\,d) = \tfrac{1}{4}\,d.$$

Their contribution to the heritable variance of b_A would equal

$$_{H_1}\sigma_{b_A}^2 = \tfrac{3}{4}\,d^2 + \tfrac{1}{4}\,d^2 - (\tfrac{1}{4}\,d)^2 = \tfrac{15}{16}\,d^2.$$

For k loci, the mean

$$M_{b_A} = \tfrac{1}{4}\,kd,$$

and the heritable variance

$$_H\sigma_{b_A}^2 = \tfrac{15}{16}\,kd^2.$$

Therefore,

$$k = \frac{15\,\Delta^2}{64_H\sigma_{b_A}^2}.$$

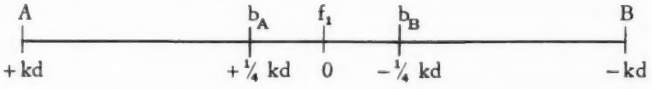
Similarly, it can be shown that

$$k = \frac{15\,\Delta^2}{64_H\sigma_{b_B}^2}.$$

Since no attempt has been made to distinguish between fixable and non-fixable heritable variance, these formulae yield minimal estimates of k. Corrections for linkage are readily applicable.

The application of these formulae requires an estimate of environmental variance ($_E\sigma^2$), since the observed variance in any generation $\sigma^2 = {}_H\sigma^2 + {}_E\sigma^2$, and ${}_H\sigma^2 = \sigma^2 - {}_E\sigma^2$, provided there is no hereditary-environmental interaction. An estimate of $_E\sigma^2$ may be obtained from replicate measurements of the parental strains. Replicate measurements of individual offspring cultures should yield the same $_E\sigma^2$ unless there is H × E interaction.

According to the formulae derived above the position of the means of the parental, f_1, and backcross generations may be plotted as follows:

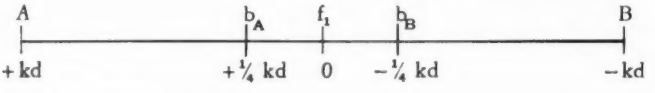


If the position of observed means are not similarly spaced, non-additivity is indicated. A suitable transformation of scale will supply the proper correction.

In organisms where all four products of meiosis are recovered, randomness of sampling may be achieved by confining f_1 measurements to one product per meiosis and backcross measurements to one product per mating. In Neurospora, this is accomplished by measuring one ascospore per ascus in the f_1 and one ascospore per mating in the backcross generations.

SUMMARY

The problem of the estimation of the number of loci governing quantitative characters in haploid organisms is discussed. Methods for the application of formulae developed for diploid organisms are developed. Formulae are derived for the estimation of the number of loci (k) directly from haploid material. The means of parents (A and B), f_1, and backcross generations (b_A and b_B) are shown to be related to each other as indicated by the following scale (where d is the contribution (+ or −) of a single allele):



The heritable variances in f_1 and backcross generations are shown to be:

$$_H\sigma_{f_1}^2 = kd^2$$

$$_H\sigma_{b_A}^2 = {}^{15}/_{16}\ kd^2$$

$$_H\sigma_{b_B}^2 = {}^{15}/_{16}\ kd^2.$$

The corresponding minimal estimates of k, where Δ is the difference of parental means, are:

$$k = \frac{\Delta^2}{4_H\sigma_{f_1}^2}$$

$$k = \frac{15\,\Delta^2}{64_H\sigma_{b_A}^2}$$

$$k = \frac{15\,\Delta^2}{64_H\sigma_{b_B}^2}$$

Application of these formulae to Neurospora is discussed.

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Dodge, B. O., 1931, Inheritance of the albinistic non-conidial characters in interspecific hybrids in Neurospora. Mycologia 23: 1–50.

Mather, K., 1949, Biometrical genetics. ix + 158 pp. New York, Dover Publications, Inc.

Wright, S., 1934, The results of crosses between inbred strains of guinea pigs differing in number of digits. Genetics 19: 537–551.

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

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Agar, W. E., 1952 (2nd edition, revised). A contribution to the theory of the living organism. 235 p. $3.75. Melbourne University Press; Cambridge University Press, New York.

Professor Agar's attempt (1943) to construct a biological philosophy in the terminology of A. N. Whitehead's metaphysical theory of organism is here reissued in slightly revised form. The essential structure of the author's original views is unaltered. A sprinkling of new references appears in the bibliography.

JOHN R. GREGG

Burns, Eugene, 1953. The sex life of wild animals. 290 p., $3.00. Rinehart and Company, Inc., New York.

Cold Spring Harbor Symposia on Quantitative Biology, the neuron, 1952. 324 p., 261 figures, 11 plates. $8.00. Biological Laboratory, Cold Spring Harbor, New York.

Twenty-six papers on structure and behavior of neurons, neuro-muscular functions, and excitation and transmission phenomena by 39 authors from the United States, Sweden, England, France, and Australia, together with discussions of the papers as presented at the 17th Symposium in June, 1952.

Droogleever Fortuyn, A. B., 1952. Age, stature and weight in Surinam conscripts. 126 p. f.2.25. Royal Tropical Institute, Amsterdam.

Measurements of 2,454 conscripts in Dutch Guiana, including those of European, Javanese, Hindostani, Chinese, and Indian descent.

Ekman, Sven, 1953. Zoogeography of the sea. 417 p., 171 fig. $6.50. Sidgwick and Jackson, Ltd., London, and The Macmillan Company, New York.

A straightforward account of the geographical distribution of marine organisms, mostly organized around Ekman's system of major regions as-

sociated with the continental shelves, and with the final chapters on the benthic and pelagic faunas of the deep seas. The faunal analyses include general summaries of ecological conditions and accounts of diverse theories of geological changes. The book thus represents a synthesis of many lines of oceanographic research and makes a rich store of knowledge of marine faunal distribution easily available to the general biologist.

Faunal and archeological researches in Yucatan caves. 1953.

Part 1. Introduction by Robert T. Hatt.
Part 2. The mammals by Robert T. Hatt.
Part 3. The birds by Harvey I. Fisher.
Part 4. The amphibians and reptiles by Dave A. Langebartel.
Part 5. The archeological findings by George W. Brainerd.
119 p., ill. $2.50. Bulletin No. 33, Cranbrook Institute of Science, Bloomfield Hills, Michigan.

Frey-Wyssling, A., 1953. Submicroscopic morphology of protoplasm. 411 pp., 181 figs., 32 tables. $8.00. Elsevier Press, New York.

In this third (second English) edition the field of submicroscopic morphology is discussed on the basis of both the indirect methods of investigation and the results obtained by means of electron microscopy since 1948. There are three divisions; the first deals with the organization of sols, the principles of structure in crystals, chemistry and gels, and with analytical methods such as X-ray analysis, polarization and electron microscopy. The second part is a comprehensive summary of our knowledge concerning the fine-structure of cytoplasm, nuclei, chloroplasts, erythrocytes and gametes. The third section treats the fine-structure of specific protoplasmic derivatives, that is, cell walls, starch grains, reserve protein and various types of fibrous proteins.

This is a valuable standard text which brings up to date our knowledge of submicroscopic structures and micellar systems forming a link between cytological and molecular configurations. Professor Frey-Wyssling emphasizes that such knowledge of morphology is a prerequisite for research into problems of physiology and development. For some reason, he draws a theoretical line between morphology and physiology, and he states that "a combination such as 'dynamic morphology' is quite inconsistent because, by definition morphology can do no more than describe or explain given spatial arrangements, whereas, as soon as *changes* in spatial arrangement are considered, we enter the domain of physiology."

Fortunately the author is more practical in his actual approach, which shows that a neat separation between morphology and physiology, between static form and dynamic change and function, is impossible, especially in an age when considerations of form and form-change enter so freely both morphological and physiological thought. Such statements as: "Special groupings in the cytoplasm, which can be designated as *morphogenetic configurations*" (page 179), "submicroscopical morphology of the hereditary

process" (page 230 ff.), "doubtless causal relations do exist between molecular morphology and morphogenesis, as foreshadowed by enzyme chemistry and the asymmetrical synthesis of organic compounds" (page 372) indicate that in the author's mind submicroscopic morphology must be anything but a static science which "can do no more than describe or explain given spatial arrangements."

ROBERT BLOCH

Grant, Madeleine Parker, 1953. Microbiology and human progress. 718 p., ill. $6.75. Rinehart and Company, New York.

This book is based on a very interesting idea—that of using microbiology as the theme for a "general education" course in biology. Microbes illustrate a wide range of biological principles and practises and student curiosity can be presupposed because of the notorious relationship between microbes and disease. Such a course would surely have more meaning for the future lawyer or businessman than the conventional introductory biology to which he is now exposed. Unfortunately this text is rather ponderous, overloaded with details about the whole catalogue of infectious diseases, and written in a dull and sometimes inept prose. It might be better, with the proposed course, to use Zinsser's "Rats, Lice and History" for the text, and have the present book for reference.

King, Thomson, 1953. Water; miracle of nature. 238 p. $3.50. The Macmillan Company, New York.

This book is written in the sort of breathless wonderment indicated by its subtitle, "miracle of nature." The first half is mostly geological and biological—water and the origins of life, and descriptions of oceans, atmospheric water, inland water, and water underground. The second half reviews water in human history (ships and pumps) and in human economy (from flood control to dowsing). There is no documentation, but the book is mostly concerned with "common knowledge" put together with a novel point of view.

Mayr, Ernst, E. Gorton Linsley, and Robert L. Usinger, 1953. Methods and principles of systematic zoology. 328 p., ill. McGraw-Hill Book Co., Inc., New York.

Mountevans, Admiral Lord, 1953. Arctic solitudes. 143 p., ill. $4.50. Philosophical Library, New York.

This is a history of arctic exploration from Pytheas to the airplane. There are scattered remarks about Eskimos, but the book contains little of direct biological interest. The photographs of Eskimo life and arctic landscapes are excellent.

Patterson, J. T., and W. S. Stone, 1952. Evolution in the genus Drosophila. 610 p., 74 figs., 109 tables. $8.50. The Macmillan Company, New York.

Given unique material at the proper moment, significant fields of research have a way of developing in an explosive fashion. With respect to the genus Drosophila, two such explosions have been witnessed in this century. The first centered around the genetics of a single species, *Drosophila melanogaster*; this resulted in the most imposing body of formal genetic knowledge yet amassed for any organism. The present book brings under one cover for the first time reviews of the newer literature on the cytogenetics and evolution of the genus as a whole, an emphasis which had its origin in the impact of formal genetics on evolutionary thought. As Drosophila is again especially suited to such studies, it is a logical development that the last 20 years should witness an extraordinarily rapid accumulation of data.

After treating the taxonomy and geographical distribution of the subgenera, species groups and species (613 of the latter are recognized), the authors present full and lucid discussions of inter- and intraspecific chromosomal and genic variability. These are followed by three chapters on isolating mechanisms and hybridization. After devoting a chapter to a detailed consideration of the peculiarly interesting case of evolution in the *Drosophila virilis* species group, the book is concluded by a chapter dealing with broad comparisons and conclusions. A 38-page bibliography is given; except in a few instances, in which more recent papers are included, the book covers the literature up through 1950.

For the non-drosophilist, the book will serve as a welcome ready reference to the already unwieldy literature on the microevolution of the genus. Although the emphasis tends to be on phylogeny rather than population genetics, each subject is handled with the balance and insight that has characterized the previous work of the authors. The specialist in Drosophila will find only minor points with which to quarrel and will be grateful to the authors for the way in which the data have been organized and integrated.

HAMPTON L. CARSON

Pettibone, Marian H., 1953. Some scale-bearing Polychaetes of Puget Sound and adjacent waters. 90 p. plus 40 plates. $3.25. (Offset). University of Washington Press, Seattle, Washington.

A valuable contribution to the taxonomy, zoogeography, and general natural history of the polychaetes. It contains an extensive bibliography; useful keys to the genera and species of the Polynoidae of Puget Sound and adjacent waters; and many good illustrations.

ACKNOWLEDGMENT

Part of the cost of publication of the recent article by Werner Braun (Amer. Nat. 86: 355–371, 1952) was covered by a grant-in-aid from the Sigma Xi-RESA research fund. The author gratefully acknowledges this aid.